A STUDY ON URINARY ISOLATES OF *CANDIDA SPECIES* ISOLATED FROM HOSPITALIZED PATIENTS WITH SPECIAL REFERENCE TO SPECIATION, ANTIFUNGAL SUSCEPTIBILITY AND COMPARISON OF RAPID AND CONVENTIONAL METHODS OF SPECIATION

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CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON URINARY ISOLATES OF *CANDIDA SPECIES* ISOLATED FROM HOSPITALIZED PATIENTS WITH SPECIAL REFERENCE TO SPECIATION, ANTIFUNGAL SUSCEPTIBILITY AND COMPARISON OF RAPID AND CONVENTIONAL METHODS OF SPECIATION"** is a bonafide record of work done by **Dr.B.Aarthy**, during the period of her postgraduate study from June 2009 to May 2012 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital,Chennai-600003 in partial fulfillment of the requirement of M.D. Microbiology degree examination of the Tamilnadu Dr.M.G.R.Medical university to be held in April 2012.

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DECLARATION

I, **Dr.B.Aarthy**, solemnly declare that the dissertation titled **"A STUDY ON URINARY ISOLATES OF *CANDIDA SPECIES* ISOLATED FROM HOSPITALIZED PATIENTS WITH SPECIAL REFERENCE TO SPECIATION, ANTIFUNGAL SUSCEPTIBILITY AND COMPARISON OF RAPID AND CONVENTIONAL METHODS OF SPECIATION"** is a bonafide record of work done by me in the Institute Of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai under the guidance and supervision of Professor, Dr.G.Jayalakshmi, M.D., D.T.C.D; Institute Of Microbiology, Madras Medical College, Chennai.

This dissertation is submitted to the TN DR.M.G.R. MEDICAL UNIVERSITY towards the fulfillment of the university regulations for the award of degree of M.D. Branch IV (Microbiology) Examination to be held in April 2012.

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Signature of the guide,
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CONTENTS

INTRODUCTION

Candida species are ubiquitous yeasts found as a part of normal flora of mucocutaneous membranes and alimentary tract of humans. They cause superficial mucosal infections usually, but can invade tissue and produce life threatening pathology caused by alteration of immune defenses.

The major medical events that caused an increased incidence of Candidial infections in the latter half of twentieth century where, i) introduction of antibacterial agents with broad spectrum of activity that alters host's normal microbial flora in favour of fungal invasion and ii)increased prevalence of immuno suppressed patients due to chemotherapy and emergence of Acquired Immuno Deficiency Syndrome[1].

Candida species can cause wide range of clinical diseases ranging from superficial infections of skin, nails and mucosal surfaces to deep seated infections involving various internal organs to disseminated disease. It can cause lower urinary tract infections and renal infection. Lower urinary tract infection (UTI) is frequently seen in association with indwelling catheters and Diabetes mellitus. Renal Candidiasis, characterized by microabcesses in cortex can occur due to hematogenous spread.

Candida spp., the most prevalent opportunistic fungal pathogens of urinary tract usually presents as nosocomial infection and rarely as community acquired infection. About 10-15% of urinary tract hospital infections are due to *Candida spp* and its prevalence is still increasing. Nosocomial UTIs were also associated with a significant attributable mortality[2,3,4].

Risk factors comprise of the extensive use of broad spectrum antimicrobial agents, corticosteroids, immunosuppressive agents, cytotoxic chemotherapy complicated by mucositis and neutropenia, elderly age ,diabetes mellitus, structural or functional abnormalities of urinary tract , indwelling urinary catheter or nephrostomy. Increased incidence is observed in Chronic Kidney Disease (CKD), patients on hemodialysis and Renal Transplant recipients due to immunosuppression[5].

Candiduria is defined as the presence of *Candida spp.* in urine and represents colonization or infection. Colonization refers to asymptomatic adherence and settlement of yeast on catheters or other foreign bodies in urinary tract. Candiduria, a marker of heavy colonization of urinary tract, correlates with invasive disease significantly more than colonization of other body sites .It should never be ignored, as it may be the only first and one of the early indications of disseminated and invasive Candidiasis, especially in critically ill patients. Candiduria was more frequent in the Candidemia due to *non-albicans Candida spp.* than that due to *Candida albicans* [6,7].

Candida albicans accounts for 40-60% yeasts isolated in developed countries, whereas Indian reports show an increased predominance of *non albicans Candida spp*. The emergence of *non-albicans Candida spp.* may represent selection of less susceptible species like *C. glabrata* and *C. krusei* . *C.glabrata* is less susceptible and *Candida krusei* is intrinsically resistant to Fluconazole. *C. tropicalis* has the highest adherence rate to inanimate materials such as urinary and vascular catheters, and is often involved in biofilm

formation, that is more resistant to Antifungal agents. Resistance to Azoles in *C. tropicalis* and *C.albicans* has also been increasingly reported [8,9,10].

There is also an increasing need for rapid speciation methods, as it can also provide information on the susceptibility to Fluconazole and hence aid in early treatment decisions.

Considering the above facts, the present study was conducted to speciate *Candida* isolated from the urine of hospitalized patients, to correlate risk factors associated and to find out the Antifungal susceptibility of the *Candida species* isolated. A comparative analysis of rapid speciation method of Chrom agar and conventional method of sugar fermentation was also carried out.

REVIEW OF LITERATURE

HISTORY

The history of *Candida species* is very old as the disease was described in ancient times. Hippocrates in his "Epidemics" written 400 B.C described "In the different ages the following complaints occur: To little and new-born children, aphthae, vomiting, coughs, sleeplessness, frights inflammation of navel, watery discharges from the ears."; the first known description of Candidal infections. Candidiasis has been recognized as a clinical entity since then[12].

Anton Von Leeeuwenhock (1632-1723), was the first to report his findings of bacteria, yeast and protozoa from scrapings from human mouth. In 1853, Charles Robin microscopically observed budding cells and filaments in epithelial scrapings, and he named the fungus *Oidium albicans*. Subsequently, more than 160 synonyms, including *Monilia albicans*, originated by Zopf in 1890, were used before *Candida albicans* became the accepted name for this species[13,14].

Berkhout in 1923 restructured the genus *Candida* to encompass these yeasts and this was accepted as the official name of the genus by Eighth Botanical Congress held in Paris in 1954.*Torulopsis*, similar fungi known by different names where brought into the domain by merging into the genus in 1978 and giving the new name *Candida glabrata* [12].

The incidence of yeast infection has increased in the last two decades. Incidence of all forms of Candidal infections has risen abruptly. The increasing incidence of Human Immunodeficiency Virus, use of therapeutic modalities for advanced support, and certain surgical procedures, such as Organ transplantation and implantation of prosthetic devices have continued to be important in expanding the incidence of *Candida* infections. These advancements have revived interest in fungal diseases and *Candida* infections in particular[15,16].

Taxonomy: Genus *Candida* belongs to

KINGDOM: FUNGI
PHYLUM: ASCOMYCOTA
SUBPHYLUM: SACCHAROMYCOTINA
CLASS: HEMIASCOMYCETES
ORDER: SACCHAROMYCETALES
FAMILY: SACCHAROMYCETACEAE

Genus *Candida* consists of 200 anamorphic species and has teleomorphs in ten different genera including *Clavispora, Debaromyces, Issatchenkia, Kluyveromyces* ,*Yarrowia* and *Pichia.* [6] Common human pathogens are,

***Candida Species* and Discoverer**	**Synonym**	**Teleomorph**
Candida albicans (C.P. Robin) Berkhout 1923 [17]	*Candida stellatoidea*[18], *Oidium albicans*[12], *Monilia albicans*[12].	-
Candida dubliniensis Sullivan et al.1995[19]	-	-
Candida glabrata Meyer & Yarrow 1978[20]	*Torulopsis glabrata*[16]	-
Candida guilliermondi	*Yamadazyma guilliermondii,Meyerozyma guilliermondii.*	*Pichia guilliermondii.*
Candida kefyr (Beijerinck) Van Uden & H.R. Buckley (1970)	*Candida pseudotropicalis*[12]	*Kluyveromyces marxianus.*
Candida krusei (Castellani) Berkhout (1923)[21]	*Candida castellani, Saccharomyces krusei* , *Endomyces krusei* , *Monilia krusei*.[22].	*Issatchenkia orientalis.*
Candida lusitaniae Van Uden & do Carmo-Sousa(1959) [23]	-	*Clavispora lusitaniae.*
Candida norvegensis	*Torulopsis norvegica.*[11]	*Pichia norvegensis*[24]
Candida parapsilosis (Ashford) Langeron and Talice, 1932	*Monilia onychopila* , *Monilia parapsilosis, Mycocandida parapsilosis.*	-
Candida tropicalis Berkhout, 1923[17]	*Candida paratropicalis* , *Candida vulgaris* , *Monilia tropicalis* , *Oidium tropicale, Mycotorula dimorpha,*	-
Candida viswanathii Sandu & H.S.Randhawa, 1962[20]	-	-

Physiology

Candida spp metabolise glucose via the Hexose Monophosphate pathway under aerobic conditions (assimilation) or via the Embden Meyerhof pathway in anaerobiosis (fermentation). Mitochondrial oxidative phosphorylation, Kreb's cycle and protein synthesis are similar to those of eukaryotic cells. *Candida* enzymes are significant as they may be directly involved in pathogenesis.

Growth temperature has an important influence on morphogenesis. Temperatures around 25°C promote the formation of chlamydospores in *C.albicans* and higher temperatures around 37°C promote formation of pseudohyphae[1].

Epidemiology

Fungi are ubiquitous in plants, mammals and insects .Humans are continually exposed to numerous genera of fungi through a variety of routes. *Candida* are normal commensals of humans and are commonly found on skin , throughout the entire gastrointestinal tract, in expectorated sputum, in female genital tract and in urine of patients with indwelling Foley's catheter[16,26].

Majority of these infections arise from an endogenous focus of colonization, human to human transmission is also possible; the documentation of nosocomial transmission or 'cross-infection' pose new and significant problems. Recent studies indicate that *Candida* may be isolated from the hands

of 15-54% of health care workers in intensive care unit setting and is involved in cross- infection [27].

Fungi represents the second most frequent causative organism of nosocomial UTI ,ranging between 12% and 27% of total pathogens .*Candida spp.* is responsible for 10-15% of nosocomial UTI. In healthy people, the prevalence of Candiduria is as low as 0-0.3% and varies in hospital settings, most prevalent in surgical ICU, leukemia and bone marrow transplant units[28,29,30].

Risk to develop Candiduria was increased by twelve-fold after urinary catheterization, six-fold after the use of broad spectrum antibiotics and presence of urinary tract abnormalities, four-fold in abdominal surgeries, two-fold in Diabetes mellitus and with corticosteroid administration. Diabetes mellitus was the most common underlying disease in almost every study of Candiduria. Candiduria is positively correlated with heavy colonization of the urinary tract. Candiduria correlates with invasive disease significantly more than colonization of other body sites[6,31,32,33].

Epidemiologic studies suggest that *non-albicans Candida spp.* are more prevalent in urine compared to other sites of *Candida* infection like oropharynx and vagina, possibly due to urine composition and or pH. However, these microbiological differences remain unexplained currently. Mixed isolates were found in 10% of patients with nosocomial Candiduria[29,32].Foreign studies on Candiduria have reported *C.albicans* as the predominant causative organism, where as Indian reports show an increased predominance of *nonalbicans*

Candida spp[31,34]. The emergence of *nonalbicans Candida spp* may represent selection of more resistant species like *C.glabrata and C.krusei*[26,35,36].

PATHOGENECITY AND PATHOGENESIS

Normal Host Response: [37,38,39,40]

- Polymorphonuclear leucocytes damages pseuohyphae, phagocytoses and kill blastospores.
- Monocytes, eosinophils and dendrocytes also ingest and kill *Candida*.
- T- helper cells regulate phagocytosis.
- Serum heat stable and heat labile opsonins increase the rate of ingestion; thereby B-lymphocytes and antibodies also have a role.
- Complement is necessary for optimal opsonisation of *Candida* blastospores.

Pathogenesis

Infectious source of *Candida* is mostly endogeneous. Gastrointestinal(GI) tract is considered to be a major reservoir for Candidiasis. Damage to GI tract can help in the hematogenous spread. *Candida* can also cross the intact GI tract by a 'persorption' and is due to fungal overgrowth due to alteration of normal balance of bacterial flora following antibiotics [1].

Candida, being a human commensal to become a pathogen, interruption of normal defense mechanism is necessary. Diabetes predisposes to cutaneous Candidiasis .Introduction of antibiotics and widespread uses of indwelling catheters are important iatrogenic factors predisposing to Disseminated Candidiasis. Other therapeutic modalities like multiple abdominal surgeries, Renal transplantation, neoplastic diseases, use of steroids and severe burns also play an important role [38].

Differential expression of virulence factors including [1,41],

Adhesins: Helps fungi to attach adhere to host tissue and form biofilms.

Morphogenesis: Reversible transition between unicellular yeast cells and filamentous growth forms helps in penetration to evade host's defence system.

Secreted Aspartyl Proteases, Phospholipases: Facilitate tissue penetration and invasion.

Phenotypic Switching: Changes in antigen expression, colony morphology and tissue affinities, helps in organism's adaptation to the hostile conditions.

Immunomodulatory effect: Contributes to reduced activity of host defense system.

PATHOLOGY

Pathological and histopathological features of *Candida* infection is highly variable, same organism can cause dissimilar pathological features in patients whose immune system is intact and those who are immunosuppressed. Pathological lesions are influenced by interaction of three factors, site of infection, pathogenicity of infecting organism and competence of host immune system [1].

SUPERFICIAL INFECTIONS:

It results from invasion of superficial layers of skin and or mucosa by the organism. Macroscopically the lesion consists of a grayish plaque, surrounded by edema, which on histopathological examination, consists of infecting organism, neutrophils and cell debris. In deep ulcerative mucosal lesions, as in immunosuppressed patients, the organism are present in the submucosa and are disseminated hematogenously .The cellular reaction is limited or absent and necrosis is accompanied by haemorrhage. Chronic mucocutaneous Candidiasis is characterized by warty proliferation, due to hyperkeratosis and epithelial hyperplasia[1].

DEEP INFECTIONS:

They are characterized by microabcesses, macroscopically they are similar to miliary tuberculosis. They contain microorganism as blastospore and or pseudohyphae, neutrophils, mononuclear cells and the abscess having a necrotic center. Granulomata and giant cells are found in chronic lesion.

Kidneys seem to be the target organ for *Candida* presenting as nephritis, pyelonephritis and rarely as papillary necrosis although liver, spleen and brain may be affected in disseminated infections. Infection may be ascending or hematogenous. Ascending infection is a sequel to prolonged catheterization and broad spectrum antibacterial therapy .It is characterized by small white lesions in medulla. When the spread is hematogenous, lesions are evenly distributed in medulla and cortex and lesions in lower urinary tract are absent [1].

CLINICAL MANIFESTATIONS:

The spectrum of diseases caused by *Candida* has increased and relatively large number of infections previously unrecognized or extremely infrequent has become well documented now. They are subdivided into mucous membrane, cutaneous and deep organ involvement [16].

Mucous membrane infection: Thrush, Candida oesophagitis, Non-oesophageal gastro-intestinal Candidiasis, Candida vaginitis.

Cutaneous candidiasis syndrome: Generalized cutaneous Candidiasis, Candida folliculitis, Erosion interdigitalis blastomycetica (*Candida* infection between fingers or toes),Candida balanitis, Intertrigo, Paronychia , Onychomycosis**,** Diaper rash, Perianal candidiasis, Chronic Muco-cutaneous Candidiasis ,Cutaneous lesions of disseminated Candidiasis.

Deep organ involvement: Central Nervous System Candidiasis, Respiratory tract candidiasis, Cardiac Candidiasis(infection of pericardium, myocardium and endocardium), Candida endocarditis, Urinary tract

Candidiasis, Candida arthritis, Osteomyelitis, Costocondritis and Myositis, *Candida* infection of peritoneum ,liver, spleen and gall bladder, *Candida* infection of vasculature, Ocular candidiasis, Neonatal candidiasis ,Syndrome of disseminated Candidiasis and Candidemia [16].

Urinary tract candidiasis:

In the recent years there has been an increased proportion of *Candida* among the nosocomial infections of which the largest increase was seen in urinary tract infections showing an upsurge from 5% to 20% .*Candida spp.* express a tropism for the kidney. Isolation of *Candida spp.* may represent a superficial or deep infection of upper or lower urinary tract, vulvo-vestibular or catheter colonization and contamination. Contamination of sample can be excluded by repeating the urine culture. Colonization is a predisposing factor to UTI and Candidemia[2,26,42].

PREDISPOSING FACTORS FOR CANDIDURIA:

Diabetes mellitus, indwelling urinary catheters, urinary tract disease, prostatic hyperplasia, stones, recurrent urinary tract infection, posterior urethral valve, antibiotics, immunosuppression ,transplant, extremes of age, female sex, radiation therapy, cytotoxic chemotherapy, neutropenia, Chronic Renal Failure and hemodialysis[43,44,45].

CANDIDURIA:

Candiduria refers to the presence of *Candida species* in urine. It is an increasingly common finding in hospitalized patients. Most patients with

Candiduria are asymptomatic and patients with indwelling catheter are more often colonized than infected with a *Candida spp*[2,46].

Ascending infection is the most common route of infection. Women are most often affected due to shorter urethra and vulvo-vestibular colonization with *Candida spp*. Urinary catheterization an important risk factor, allows direct introduction or migration of organisms into bladder along the surface of catheter. All catheters become colonized if left in-situ for a longer time. *Candida*'s growth in urine is enhanced when urinary level of glucose exceeds 150mg/dl[2,44,47].

In contrast, hematogenous spread is one of the most important route for renal candidiasis. This might be of outstanding importance because 90% of patients with Candidemia who have died, had autopsy proven renal involvement[48].

Unfortunately, colonization versus infection, lower urinary tract versus upper urinary tract involvement and hematogeneous versus ascending infection are very difficult to differentiate clinically. Pyuria is not predictive of infection, it can be explained by mechanical injury of bladder mucosa by urinary catheter and is frequently the result of coexistent bacteriuria[30,49].

Candiduria can be Asymptomatic, Lower Candidal UTI, Candidal Cystitis and Upper Candidal UTI. Patients with Candiduria are at potential risk for Invasive Candidiasis. It has been shown that 96% of patients are asymptomatic and only 4%, complain of symptoms. No diagnostic tools are available to readily differentiate between Candidial UTI and colonization [28,50].

Candiduria seems to correlate with invasive disease significantly, where as absence of *Candida* in urine has nearly 100% negative predictive value for systemic infection [6].

LAB DIAGNOSIS OF CANDIDURIA

Specimen collection: It has to be done under strict aseptic precautions, as it can become easily contaminated with bacteria from vaginal canal, perineum or indigenous flora in the urethra [51].

Specimens are to be collected in a wide mouthed sterile, leak-proof screw capped container. Specimens collected are mid-stream urine specimens, catheter collections, suprapubic aspiration and straight catheterized urine. Suprapubic aspiration is reserved almost exclusively for neonates and small children , a small incision is made with an 18 gauge needle over suprapubic skin and 10ml of urine is aspirated into syringe [51].Straight catheterized urine is more invasive and as urethral organisms can be introduced into the bladder with catheter, this procedure is often not preferred [52]. Mid-stream urine and catheter collections are the common specimens in adult patients.

Direct microscopic examination: It is highly useful for preliminary screening .It is done on a wet film in high power objective .Wet mount can be prepared. Gram's stain of centrifuged urine is also done. Microscopic examination will reveal budding yeast cell, pseudohyphae and leucocytes. Yeast cells are approximately 4-8µm with budding and pseudohyphae[12,54].

Cultural characteristics: Isolation of yeast is done by culturing on Sabouraud's Dextrose Agar (SDA) with Chloramphenicol , Gentamicin and or Tetracycline at 25°C and 37°C.It should be incubated for 48hrs, before a report of no growth is given. They appear pasty, opaque, pale or white or off-white or beige colored, often with a sweet smell reminiscent of ripe apples. A wet mount and Gram's stain should be made from cultures when colonies appear [1,12,54,55].

Chrom Agar: A chromogenic differential culture medium is being used to facilitate isolation and presumptive identification of clinically important yeast species, especially *Candida albicans*.It is a rapid, plate based method for simultaneous isolation and identification of species. It is based on direct detection of specific enzymatic activities by adding certain substrates of flurochromes to the media [12].

Corn-Meal Agar or Corn-Meal Tween 80 Agar: Cornmeal-tween 80 agar enhances the production of characteristic microscopic structures such as chlamydospores. Dalmau plate culture is used to observe them. A heavy inoculum of yeast is streaked across the plate containing medium and coverslip is placed over it. The streak should extend beyond the cover slip. Plates are incubated for 48hrs at 25°C.The edge of the coverslip is observed under low power objective as chlamydospores are seen maximum in this area.

These are helpful in presumptive identification, differentiating organisms with identical biochemical profiles and as quality control check of species identification in automated kits[12,51,55].

Germ-tube test:A portion of yeast colony is inoculated into 0.5 ml of rabbit or human plasma or serum and incubated at 35ºC for 2 hours. A drop of yeast and serum suspension is placed on a microscope slide, overlaid with a coverslip and seen for the presence of germ tubes .Germ tube is a filamentous extension and it is half the width and three- four times the length of the mother cell. No constriction is seen where the germ tube emerges from the yeast. Germ tubes are formed in *C.albicans* and *C.dubliniensis*. Not all strains of *C.albicans* produce germ tube. The procedure is used for presumptive identification of *C.albicans* .The demonstration of germ tube is known as Reynolds Braude phenomenon [12,51,55].

Biochemical tests: The genus *Candida* and different *Candida spp.* can be characterized by the pattern of utilization of carbohydrate and nitrogen substances. *Candida spp.* can utilize carbohydrates oxidatively called assimilation and anaerobically called fermentation.

Carbohydrate fermentation: Liquid medias are supplemented with different carbohydrates,a colour indicator to detect pH changes due to acid production and Durham's tube to detect gas production[1].

Carbohydrate Assimilation: The classical Wickherham and Burton method assessed the ability of the yeast isolate to grow in a minimal liquid media supplemented with carbohydrates. As it was laborious and time consuming, it was replaced by auxanographic technique of Hazen and Howell in 2003, in which minimal media agar plates, Yeast Nitrogen Agar Base (YNB without a carbohydrate source) on which paper discs impregnated with different carbohydrates are placed. Assimilation can be determined by the

ability of the yeast to grow around carbohydrate disc. Commercial systems to determine assimilation of 20 carbon substrates have now been introduced [1,56].

Nitrate assimilation test: Yeast carbon agar with addition of various nitrogen sources tests the ability of yeast to assimilate nitrogen. Peptone and potassium nitrate are used as nitrogen test sources. It can be determined by the ability of the yeast to grow around potassium nitrate and peptone disc in Yeast Carbon Agar [57].

Immunodiagnosis:

Detection of Candida antigens: *Candida* antigens like mannans, cytoplasmic antigens like enolase and glycoprotein can be detected by Enzyme ImmunoAssays, Radio ImmunoAssay and Latex agglutination tests using monoclonal antibodies [56].

Detection of Candida antibodies: Antibodies to *Candida* antigens released or expressed during deep seated infection like enolase and heat shock proteins, can be determined by Immunodiffusion(ID), Counterimmune electrophoresis (CIE), Enzyme Linked Immune Sorbent Assay(ELISA) or Latex agglutination(LA) test. Low sensitivity occurs due to a less sensitive test or due to poor immune status of patients. Antimannan antibodies are normally found in healthy individuals and superficial infection [56].

Other methods: [1, 12]

Detection of Proteinase : Auxanographic methods.

Detection of Fungal Metabolites: Fungal metabolites like D-arabinitol and D-mannose in serum and urine are detected by Gas-Liquid Chromatography & Enzymatic fluorometric assessments.

Molecular Techniques: It includes PCR using DNA probes or rRNA probes, Restriction fragment length polymorphism, Southern hybridization pattern, Electrophoretic patterns of DNA,RNA profiling.

ANTIFUNGAL SUSCEPTIBILITY TESTING:

It has become important in the Clinical Laboratory due to

i. The increased incidence of fungal infections during the last 15 yrs,

ii. The more frequent and prolonged use of both established and investigational Antifungal agents;

iii. The increased awareness of the emergence of resistant organisms to established Antifungal agents; and

iv. The need to develop a reliable database to document the relationship between the in vitro result and the clinical outcome of therapy to define ultimate utility of Antifungal Susceptibility testing[58].

Standardization of in vitro susceptibility tests by the Clinical Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) were highly useful[59].

BROTH BASED METHODS

1. **Macrobrothdilution method**: This was the first method proposed by CLSI.As it was too cumbersome, it was replaced by Microbroth Dilution method. M27-A2 standard is the document intended for testing yeasts by using either a Macro or MicroBroth Dilution test system.

2. **Microbroth dilution:** It recommends the use of RPMI-1640 medium (with glutamine, phenol red, without bicarbonate) supplemented with 0.2% glucose and buffered to a pH of 7.0 with 0.165 mol/L MOPS buffer. Antifungal drugs are added to the wells in serial dilutions as recommended by CLSI. Inoculum standardized to 0.5 McFarland's is added and incubated at 35°C. Plates are read at 24 hours. The wells should be visualized with a reading mirror and the growth in each well should be compared with that of the growth control. The MIC for Amphotericin B is lowest concentration which is optical clear and for Azoles, 5-Fluorocytosine MIC are lowest concentrations with a prominent reduction in turbidity [60].

3. **Spectrophotometric method**:It is a more objective measurement of yeast growth than subjective visual assessment, and affords a simpler ,more reliable basis for data analysis than visual scoring of turbidities on a 1+ to 4+ scale. This method provides a better reproducibility, good agreement with CLSI method [61].

4. **Sensititre Yeast One Test Panel /Fungitest:** A MicroBroth Dilution method based on the CLSI M27-A2 standard. It consists of a disposable microtitre plate with dried serial dilutions of Antifungal agents in wells. The wells contain Alamar Blue, a redox indicator as colorimetric

indicator. End point is read by a colour change from blue to pink. MIC results when compared with CLSI method showed favorable results [62,63,64].

5. **Flow Cytometry** : Assay for Antifungal Susceptibility testing by measuring the impairment of fungal metabolic activity. Yeast viability analyzed by Flow Cytometry with a fluorescent probe, FUN-1, which emits a red fluorescence when yeast is metabolically active. This method yielded reliable results[65].

AGAR BASED METHODS

1. **Disk diffusion:** The M44-A standard is a newly established methodology for disk diffusion testing of *Candida species*. The standard includes zone interpretive criteria for Fluconazole and Voriconazole. It recommends the use of Mueller-Hinton Agar supplemented with 2% glucose and 0.5 ug/ml methylene blue dye medium. Mueller-Hinton agar is readily available, with acceptable batch-to-batch reproducibility, glucose provides a suitable growth for most yeasts and the addition of methylene blue enhances the zone edge definition. Inoculum is standardized to 0.5 McFarland's and plates should be incubated at 35 ºC for 24 hours [60].

2. **Etest**: An agar diffusion method using a strip with a predefined concentration gradient of the antimicrobial agent being tested, that allows for MIC determination. It is done on modified RPMI-1640 agar supplemented with 0.2% glucose. Etests are simple to perform with

reasonable correlation to CLSI standard. Resistant isolates need to be confirmed by CLSI methodology [67,68].

3. **Neo-Sensitabs**: A simple agar diffusion method using tablets to determine the susceptibility of fungi with a Biomic plate reader to electronically read and interpret zone sizes. Resistant isolates need to be confirmed by CLSI methodology [63,64].

TREATMENT OF CANDIDURIA

Asymptomatic Candiduria:

- Risk factors are to be modified, which includes removal of catheter, control of Diabetes and discontinuation of antibiotics.
- It is treated in Renal transplant recipients , low-birth weight infants ,patients who have or had neutropenia and prophylaxis in patients undergoing invasive urological procedure as follows,

  Fluconazole 200mg/day orally for 7-14 days or

  Amphotericin B, 50mg/l for bladder irrigation or 0.3mg/kg IV or

  5-Flucytosine, 150mg/kg/day for 7-14 days.

Candida cystitis:

- Fluconazole 200mg/day orally for 7-14 days.

Ascending pyelonephritis:

- Surgical drainage,

- Fluconazole 6mg/kg/day for 2-6 weeks,
- Amphotericin B IV >0.6mg/kg /day or Caspofungin 50mg/day for 2-6 weeks.

Renal candidiasis:

- Fluconazole 6mg/kg/day for 2-6 weeks,
- Amphotericin B IV > 0.6mg/kg /day or Caspofungin 50mg/day for 2-6 weeks[2,3].

ANTIFUNGAL RESISTANCE: [26]

It is divided into (i) Clinical resistance and (ii) Cellular or In-vivo resistance.

I) Clinical Resistance, is a result of low level of drugs in serum and or tissues caused by poor patient adherence, drug interaction that decreases antifungal levels and in patients with severe immuno-suppression like AIDS.

II) Cellular or In-Vivo Resistance, is independent of host and involves strains less responsive to Antifungal agents at standard doses .It can be primary or secondary resistance. Primary or intrinsic resistance is demonstrated by organisms that are naturally resistant to Antifungals. Examples include *C.krusei* intrinsically resistant to fluconazole, replacement of original isolate by more resistance strain or more resistant species, genetic alteration resulting in a more resistant strain

and transient gene expression resulting in a temporary resistance. Secondary or acquired resistance occurs when initially susceptible isolate becomes resistant to an Antifungal agent .It is commonly encountered in HIV positive patients.

Mechanisms of resistance: [26]

Azole resistance: It is due to

- Changes in sterol components of plasma membrane,
- Genetic changes in ERG11 gene encoding Lanosterol demethylase,
- Alteration of enzymes involved in Ergosterol biosynthesis and
- Drug efflux mechanisms.

Amphotericin B resistance: Poorly understood, it is probably due to changes in sterol components of plasma membrane and lipid composition of cell membrane.

Flucytosine resistance: Deficiency of cytosine permease, cytosine deaminase, UMP pyrophosphorylase and loss of feedback regulation leading to increased synthesis of pyrimidines.

Echinocandin resistance : Due to mutations in FKS genes encoding 1,3 β-D glucan synthase.

AIMS AND OBJECTIVES

- To speciate the *Candida* isolates from urine of hospitalized patients.
- To find out the ratio of *Candida albicans* to *non-albicans Candida species.*
- To correlate the risk factors to the *Candida species* associated.
- To compare the speciation of *Candida* isolates using the rapid technique of Chrom agar with the conventional techniques of sugar fermentation and sugar assimilation tests.
- To find out the antifungal susceptibility of the *Candida species* isolated.

MATERIALS AND METHODS

- **Design of the study:** Cross-sectional study.

- **Study period:** Oct 2010 –Sept 2011**.**

- **Place of Study:** Institute of Microbiology, Madras Medical College **&** Rajiv Gandhi Government General Hospital, Chennai.

- **Ethical considerations:** Approved by Institutional Ethics Committee, Madras Medical College **&** Rajiv Gandhi Government General Hospital, Chennai.

- **Statistical analysis:** Statistical analysis was carried out using statistical package for social sciences and episoftware by Statistician. The proportional data were tested using Pearson's Chi Square Analysis test and Fischer's exact test.

- **Study group:** Study group included 100 hospitalized patients with Candiduria having a colony count of more than 10^4/ml of urine.

Inclusion criteria:

- Hospitalized Patients with urine colony count of any *Candida species* more than or equal to 10^4/ml.

- Patients older than 12 yrs of age.

- Both males and females were included.

Exclusion Criteria:

- Candiduria with colony counts less than 10^4/ml of urine.
- Patients less than 12 yrs of age.
- Outpatients.

Patients satisfying the inclusion criteria were included in the study and after getting informed consent, they were assigned serial numbers. They were interviewed by structured questionnaire and their hospital records were used to know about the history, risk factors, duration of Candiduria and treatment details.

SPECIMEN COLLECTION

Urine Specimens were collected in a wide mouthed, sterile, leak-proof screw capped container. Specimens collected were mid-stream urine specimens, and catheter collections. They were immediately transported to laboratory without any delay, as urine is an excellent culture medium. Delay of more than 1-2 hr in transportation if unavoidable, it should be stored in refrigerator at 4°C or transported in a container with 1.8% boric acid [54].

Mid-stream urine specimen: Urine samples are commonly collected by this technique. The peri-urethral and perineal areas are first cleansed with gauze pads soaked in soapy water. The labia should be held apart during voiding in females and in males, the foreskin is retracted, if uncircumcised .The first few milliliters of urine passed into a bed pan or toilet bowl to flush the bacteria from urethra. The mid-stream portion is then collected into the container [51,68].

Catheter Collections:The area is disinfected before proper collection of samples. Urine samples are aspirated using a sterile syringe and needle (gauge no.28), through a soft rubber connecter between catheter and collecting tubing. Urine samples should not be collected from catheter bags and catheter tips are also not suitable for culture as they can be contaminated with urethral or colonizing organisms [51,69].

SPECIMEN PROCESSING:

Direct microscopical examination:

Microscopic visualization of uncentrifuged urine was done. One to three *Candida* per high power field was found to be equivalent to colony count of approximately 15000/ml with 80% accuracy. A wet mount of uncentrifuged urine was done. A small volume of urine was applied to a glass microscope slide, allowed to air dry, stained with Gram's stain, and examined microscopically for presence of gram positive budding yeast cells with or without pseudohyphae[1,70].

Each patient's urine was transferred into sterile centrifuge tubes and then centrifuged at 3000rpm for 10-15 minutes. The supernatant was discarded and the deposit examined microscopically by Gram's stain for yeast cells, budding cells and pseudohyphae [71, 72].

Culture: [12,73,74]

- The uncentrifuged mid-stream urine was cultured on blood agar, Mac Conkey agar, Cystine lactose electrolyte deficient (CLED) agar and Sabouraud's Dextrose Agar (SDA) for primary isolation of *Candida spp*.

- All the plates were incubated at 37°C .SDA was incubated at 25°C and 37°C. The plates were read at 24 and 48 hrs after incubation.

- Colonies were cream colored, pasty and smooth. A wet mount, lacto phenol cotton blue (LPCB) mount and Gram's stain were done from the culture isolates to confirm it as *Candida species.*

CANDIDA SPECIES	**MACROSCOPIC FEATURES**[55]
C.albicans and *C. dubliniensis*	Colonies are cream coloured, pasty and smooth.
C.tropicalis	Colonies are cream to off-white in colour, glistening to dull,soft, smooth or wrinkled with a mycelia fringe.
C. kefyr	Creamy , dull ,soft and smooth colonies.
C. krusei	Flat, dull, dry and wrinkled colonies.
C. parapsilosis	Creamy colonies developing in a lacy pattern.
C.guillermondii	Thin ,flat, glossy,cream to pink, smooth or wrinkled colonies.
C. glabrata	Glistening, smooth, cream coloured colonies.

CANDIDA SPECIES	**MICROSCOPIC FEATURES**[55]
C. albicans ,*C.parapsilosis*	Ovoid,elliptical or round blastoconidia seen .
C. guillermondii ,*C. tropicalis*	Ovoid or elliptical blastoconidia seen.
C. kefyr ,*C. krusei*	Elongated, slender oval blastoconidia seen.

Colony count:

- Colony counts were carried out using 10-fold serial dilutions of uncentrifuged urine in normal saline, ranging from 1 in $10^1$to 1 in 10^6. Urine samples were spread by calibrated loops onto Sheep blood agar.

- Loop was touched in the centre of plate from which the inoculum was spread across the diameter of the plate. Loop was then drawn across the entire plate, crossing the first inoculums streak numerous times to produce isolated colonies.

- Dilution factors and loop factor were then multiplied to get the final colony count per ml of urine[52,73,75].

SPECIES IDENTIFICATION: The various *Candida spp.* were identified based on the following tests.

Germ tube test:

- A small portion of an isolated colony was suspended in a test tube containing 0.5 ml of rabbit or human plasma or serum.
- The test tube was incubated at 37°C for 2 hours.
- A drop of yeast suspension was placed on a microscope slide, overlaid with a coverslip and examined microscopically for the presence of germ tubes which are long tube like projection from yeast cells.
- Isolates producing germ tubes were presumptively identified as *C.albicans* or *C.dubliniensis*[12,51,54].

Growth at 42°C:

- Germ tube positive isolates were subcultured to SDA and incubated at 42°C.
- Isolates of *C.albicans* grow, while *C.dubliniensis* do not grow at 42°C [76].

Surface growth:

- A colony was inoculated into Sabouraud's Dextrose broth and incubated at 30°C for 48hrs.
- *C.tropicalis,* produces a pellicle at the broth surface.

- *C.krusei,* grows as a climbing film on the sides of the glass[54].

Candida HI-CHROM agar:

- Yeast isolates were subcultured twice onto Sabouraud's Dextrose Agar to ensure isolation of pure colonies.

- Isolates were plated directly from SDA to HI-Chrom agar.

- The plates were then incubated at 30°C for 48 hours. Color readings were made after 48 hours of incubation.

- The various species of *Candida* were identified by their colony color, size, texture, and presence of color diffusion into the surrounding agar presumptively in 48hrs[12,49,77,78].

Candida species	**Colour on HI-Chrom agar**
C. albicans	Light green colour colonies.
C. dubliniensis	Dark green colonies.
C. tropicalis	Steel blue colonies with a pink halo.
C. glabrata	Pink to Purple colonies.
C. parapsilosis	Cream colored colonies.
C. guilliermondii	Cream to Pale pink or purple colonies.
C. krusei	Pale pink, dry rough colonies with spreading, pale edges.

Corn meal agar (Dalmau plate culture technique):

- Inoculating loop was loaded with test organism from primary culture media.
- Three parallel cuts were made 1/4inches or 1 cm apart on the surface of Cornmeal agar, by holding the inoculating wire at 45 degree angle.
- A cover-slip was laid on the surface of agar, covering a portion of inoculated streaks.
- Plates were incubated at 30°C for 24-48 hours in moisturized chamber.
- Plates were examined by placing it in a microscope whose slide carrier is removed and pattern of growth like Chlamydospores, hyphae, pseudohyphae and their relationship was observed.

MORPHOLOGY ON CORNMEAL AGAR

CANDIDA SPECIES	
C. albicans	Blastoconidia, pseudohyphae and large, thick walled, terminal chlamydospores present.
C. guilliermondii	Blastoconidia at constrictions of pseudohyphae.
C. dubliniensis	Terminal chlamydospores are abundant.
C. tropicalis	Ovoid blastoconidia anywhere along pseudohyphae.
C. krusei	Chains of blastoconidia at junction of pseudohyphal cells, with a characteristic 'crossed matchstick' appearance.
C. parapsilosis	Short,curved pseudohyphal cells develop into giant cells.
C. kefyr	Elongated blastoconidia at junction of pseudohyphal cells, separate from pseudohyphae and lie parallel to it, with a "log jam" arrangement.

It is helpful in making a presumptive identification and is not confirmatory. Chlamydospores production by *C.albicans* is diagnostic [51,54,55].

Sugar fermentation test:

- A suspension of pure colony of yeast from sugar free media was made in sterile distilled water equivalent to McFarland's opacity standard of 4 and 0.2ml of suspension was inoculated to a 2% sugar fermentation media with an indicator.

- Dextrose, sucrose, lactose and maltose were tested.

- They were incubated at 48-72hrs at 30°C and further incubated up to 1 week.

- The ability to ferment sugars was identified by presence of acid production and gas trapped in durham's tube.

Fermentative yeasts produce carbon dioxide and alcohol .pH may not change and therefore gas production is itself indicative of fermentation [54,79,80,81,82].

Sugar assimilation test/ Auxanographic Procedures

- The medium yeast nitrogen base (YNB) without the carbohydrate source was used. Agar was autoclaved at 121°C.

- YNB was added at 6.7gm/100ml of distilled water and sterilized by filtration.

- Heavy yeast suspension made from two to three colonies of the 24-48 hr culture grown in a sugar free media was added to 2ml of YNB prepared . It was emulsified to turbidity equal to McFarland's 4 units.

- The YNB & yeast suspension was added to 18ml of molten agar cooled at 45°C, gently swirled to distribute the yeast cells in the molten agar, and the entire volume was poured into a 90mm petri plate.

- Sugar discs should be placed in a circle with sterile forceps, such that at least 30mm was present between centers of each disc and incubated at 30°C for 24-48 hrs.

- If the sugar was assimilated, a zone of growth appears around the disc [81,83,84].

ANTIFUNGAL SUSCEPTIBILITY TESTING:

Antifungal Susceptibility Testing for *Candida* isolates was done by,

- Disc diffusion method, as per CLSI Guidelines on Antifungal Susceptibility testing in M-44A document.

- Microbroth dilution technique as per CLSI Guidelines on Antifungal Susceptibility testing in M-27A2 document.

Disc diffusion method:

Medium: Mueller Hilton Agar with 2% glucose and 0.5 μg methylene blue dye adjusted to a pH of 7.2-7.4was used.

Inoculum preparation:

- Organisms were subcultured and incubated at 35°C to ensure purity and viability.
- Inoculum was prepared by direct colony suspension method. Five colonies of approximately 1 mm diameter from a 24-hour old culture was picked and suspended in 5 ml of sterile normal saline.
- Suspension was vortexed and adjusted spectrophotometrically at 530 nm to 0.5 McFarland's standard containing, 1x 10^6 to 5 x 10^6 cells/ml.

Inoculation of test plate

- Sterile cotton swab stick was dipped into suspension, rotated several times and pressed firmly against the inside wall of the tube above fluid level to remove excess fluid.
- It was streaked evenly over the entire agar surface 3 times, rotating each time at an angle of 60°C, to ensure an even distribution of inoculum.

Application of disks to inoculated plates

- Antimicrobial discs were dispensed onto the surface of an inoculated agar plate by means of a sterile forceps and pressed down.

- The discs were evenly distributed on the plate with a distance of 2.5 cm from centre to centre of the discs.

- Plates were inverted and incubated at 37°C within 15 minutes.

Reading and Interpretation: [81,85]

- Results were read at 20-24 hours, when semi-confluent growth had formed. In case of insufficient growth, it was read at 48 hours.

- Zone of inhibition was measured at the point where there is prominent reduction in growth.

- QC strains used to ensure quality control were ,

Candida albicans **ATCC 90028 and** ***Candida tropicalis*** **ATCC 750.**

Zone diameter, (nearest whole in mm)

Antifungal agents, Disc content	**Resistant**	**Susceptible – Dose Dependent**	**Sensitive**
Fluconazole,25µg	<14mm	15-18mm	>19mm
Itraconazole,10µg	< 11mm	12-19mm	>20mm
AmphotericinB,100U	<10mm	Not applicable	>10mm

The interpretive criteria for the Fluconazole disk test were those published the CLSI guidelines M44. Break points for Itraconazole (10µg) and Amphotericin B (100U) are not available. The response was interpreted

according to the study by Negri et al [87].A quality control was performed in all batches in accordance with the CLSI document M27-A2 by using QC strains.

Microbroth dilution technique:

Antifungal agents: They were obtained from reputed company, Pharma-Fabricon as pure salt, with details of potency as µg /international units per mg of powder.

Standard solution of an antifungal agent can be prepared by calculating the weight of the powder by, Target volume of stock solution (mg) X Desired concentration (µg/ml) /Potency(µg/mg).

Volume of Solvent is calculated by, Weight of Powder (mg) X Potency(µg/mg) /Desired concentration(µg/ml).

Solvents for preparing stock solutions of Antifungal agents include, Dimethylsulfoxide (DMSO) for Amphotericin B and Itraconazole and water for Fluconazole.

- For Antifungal agents soluble in water, stock solutions were prepared at ten times the highest concentration to be tested.
- For Antifungals insoluble in water, stock solutions were prepared at hundred times the highest concentration to be tested.
- A series of dilution at 10 or 100 times final dilution were also prepared to avoid dilution artifacts due to precipitation of compounds.

- For Amphotericin B ,Itraconazole the drug concentration ranged from 0.0313 to 16 µg/ml so stock solution was prepared from 03.13 to 1600 µg/ml.

- For Fluconazole, the drug concentration ranged from 0.125 to 64 µg/ml so stock solution was prepared from 1.25 to 640 µg/ml.

Broth medium and buffer:

RPMI 1640 with glutamine, without bicarbonate with phenol red as indicator was the media used. It was buffered to pH 7.0 at 25°C with MOPS ,3-N-morpholino propane sulphonic acid buffer to a final concentration 0.165 mol/l .

Inoculum preparation:

- All isolates were subcultured twice into SDA at 35°C to ensure purity and viability.

- Inoculum was prepared by picking up 5 colonies of <1mm diameter from a 24 hr old culture and suspended in 0.85% saline. The resulting suspension was vortexed and cell density was adjusted to 0.5 Mc Farland's standard at 530nm with a spectrophotometer. This yielded about $1X10^6$ to $5X10^6$ cells/ml.

- A working suspension was made by 1 in 1000 dilution with RPMI 1640 broth medium.

- This inoculum was diluted 1:1, then wells were inoculated to achieve a final desired inoculums size with a final concentration of $5X10^2$ to $2.5X10^3$ cells per ml.

Procedure:

- The test was performed in a sterile, disposable,96 well microdilution plate using standard RPMI 1640.

- From the various dilutions of stock solution (10 X drug concentration), working dilutions were prepared at 2 X drug concentration with RPMI1640 and was dispensed into the wells of row 1 to10 of the microdilution plate using a pipette. Row 1 contains the highest drug concentration and row 10 contains the lowest drug concentration.

- Each well was inoculated with 100µl of 2 X final inoculum suspension, which brings the inoculum and drug dilutions to final concentration.

- Growth control well contains 100µl of sterile drug free medium with 100µl of 2 X inoculum suspension.

- Row 11 was used to perform the sterility control containing the drug free medium only.

- Quality control strains were also tested. Plates were incubated at 35° C for 48 hrs.

Reading and Interpretation:

MIC was detected by the amount of growth in the well containing the agent was compared with the amount of growth in the control well. A numerical score from 0 to 4 was given to each well using following scale [12] ,

Score 0	Optically clear.
Score 1	Slightly hazy.
Score 2	Prominent reduction in turbidity.
Score 3	Slight reduction in turbidity.
Score 4	No reduction in turbidity.

Amphotericin B:

MIC was read as the lowest drug concentration in which a score of 0, optically clear was observed.

Azoles:

MIC was read as the lowest drug concentration in which a score of 2 with prominent decrease in turbidity was observed. A slight turbidity can occur above the MIC and is identical for all drug concentration above the MIC. Allowable turbidity was estimated by diluting 0.2ml of drug free growth control with 0.8ml of media, producing an 80% inhibition standard[12,60].

INTERPRETIVE CRITERIA FOR MICROBROTH DILUTION TECHNIQUE[60]

Antifungal agents, range of dilutions used in MBD	**Sensitive**	**Susceptible – Dose Dependent**	**Resistant**
Fluconazole, 0.125 to 64 µg/ml	< 8 µg/ml	16-32 µg/ml	>64µg/ml
Itraconazole, 0.0313 to 16 µg/ml	< 0.125 µg/ml	0.25-0.5 µg/ml	>1µg/ml
Amphotericin B, 0.0313 to 16 µg/ml	<1µg/ml	Not applicable	>1 µg/ml

RESULTS

This cross-sectional study was carried out during the period of October 2010 to September 2011 in the Institute Of Microbiology, Madras Medical College, Chennai. Speciation of isolates from 100 hospitalized patients with Candiduria was done and their Antifungal Susceptibility testing was done by Disk Diffusion and Microbroth Dilution technique.

Table 1

AGE DISTRIBUTION OF THE STUDY POPULATION

Age (Years)	Total no of cases n=100 no (%)	Male n=56 no (%)	Female n=44 no (%)
<20	4(4%)	3(5.4%)	1(22.7%)
20-29	19(19%)	6(10.7%)	13(29.5%)
30-39	15(15%)	7(12.5%)	8(18.2%)
40-49	19(19%)	10(17.9%)	9(20.5%)
50-59	16(16%)	12(21.4%)	4(9.1%)
>60	27(27%)	18(32.1%)	9(20.5%)
Total	100(100%)	56(100%)	44(100%)

Majority of the study population belonged to the above 60years age group (27%), followed by (19%) patients in 20-29 & 40-49 years age group. Females predominated in the age group of 20-29 yrs (29.5%), where as males predominated after 60 yrs (32.1%).

Table 2

TYPE OF PRESENTATION OF CANDIDURIA IN MALES AND FEMALES IN VARIOUS AGE GROUPS

Type of presentation, n(%)	Age Group(Years)											
	<20		20-29		30-39		40-49		50-59		>60	
Symptomatic patients 27 (27%)	T =1(1%)		T=6(6%)		T=2(2%)		T=3(3%)		T =3(3%)		T=12(12%)	
	M 1 (1%)	F -	M 2 (2%)	F 4 (4%)	M 1 (1%)	F 1 (1%)	M 2 (2%)	F 1 (1%)	M 2 (2%)	F 1 (1%)	M 11 (11%)	F 1 (1%)
Asymptomatic patients 42(42 %)	T =1(1 %)		T =4(4%)		T =8(8%)		T =10(10%)		T =11(11%)		T=8(8%)	
	M 0 (0%)	F 1 (1%)	M 2 (2%)	F 2 (2%)	M 4 (4%)	F 4 (4%)	M 5 (5%)	F 5 (5%)	M 9 (9%)	F 2 (2%)	M 4 (4%)	F 4 (4%)
*Unconscious patients 31(31 %)	T =2(2%)		T =9(9%)		T =5(5%)		T =6(6%)		T =2(6%)		T=7(7%)	
	M 2 (2%)	F -	M 2 (2%)	F 7 (7%)	M 2 (2%)	F 3 (3%)	M 2 (2%)	F 4 (4%)	M 1 (1%)	F 1 (1%)	M 4 (4%)	F 3 (3%)
Total 100	4(4%)		19(19%)		15(15%)		19(19%)		16(16%)		27(27%)	

T-Total,M-Male,F-Female

Most of the patients had asymptomatic Candiduria (42%), followed by symptomatic patients (27%) and Unconscious patients who were unable to tell the symptoms (31%). Symptomatic and unconscious patients were common in the age group of > 60yrs, were as asymptomatic patients were common in the age group of 40-49yrs and 50-59yrs.

Table 3

PRESENTATION OF VARIOUS SYMPTOMS IN PATIENTS WITH CANDIDURIA

SYMPTOMS	Male, n=56,no (%)	Female, n=44, no (%)	TOTAL, n=100 no (%)
Fever	12(21.4%)	4(9.1%)	16(16%)
Dysuria	9(16%)	2(4.5%)	11(11%)
Increased frequency	7(12.5%)	2(4.5%)	9(9%)
Urgency	7(12.5%)	1(2.3%)	8(8%)
Lower abdominal pain	6(10.7%)	1(2.3%)	7(7%)
Incontinence	5(8.9%)	1(2.3%)	6(6%)
Back pain	2(3.6%)	3(6.8%)	5(5%)
Urinary retention	5(8.9%)	0(0%)	5(5%)

Among the patients with Candiduria fever (16%), dysuria (11%) were the predominant symptoms. Symptoms of frequency,urgency were present in 9%, 8% respectively and lower abdominal pain in 7% of patients. Dysuria, increased frequency and lower abdominal pain were predominant in males than in females.

Table 4:

INFECTION IN SYMPTOMATIC PATIENTS WITH CANDIDURIA

DISEASES	**No (%)**
Pyelonephritis	5(18.5)
Cystitis	3(11.1)
Lower UTI	19(70)
Total, n	27(100)

Lower UTI was common in 19(70%), followed by Pyelonephritis in 5(18.5%) and Cystitis in 3(11.1%) of the symptomatic patients with Candiduria.

Table 5

WARD DISTRIBUTION & THE RISK FACTORS IN PATIENTS WITH CANDIDURIA

WARD, number of patients	Major risk factor associated & no (%)
Medicine, n=35	Diabetes mellitus-26(74) Antibiotic -25 (71) Catheterization-22(63)
Nephrology, n=17	CKD & diseases of urinary tract-17(100) Antibiotics-14(82)
ICU(Intensive Care Unit), n=12	Antibiotic-12(100) Catheterization -12(100)
Urology, n=10	CKD, other diseases of urinary tract-10(100) Catheterization-10(100) Antibiotic -8(80)
Surgery, n=14	Antibiotic-13(93) Catheterization-13(93)
Neurosurgery, n=6	Catheterization-6(100) Prolonged antibiotic -6(100) Immunosupressives (steroids)-5(100)
Orthopedics, n=3	Catheterization-3(100) Prolonged antibiotic -3(100)
Geriatrics, n=2	Catheterization-2(100)
Rheumatology, n=1	Catheterization-1(100) Diabetes mellitus -1(100) Immunosupressives(steroids)-1(100)

Patients from Medical ward had Diabetes mellitus as a major risk factor, were as patients from Urology and Nephrology had diseases of urinary tract as the major risk factors.ICU and Surgery, Orthopedics, Neurosurgery patients had antibiotic use and catheterization as major risk factor .In addition, immunosuppressives were a major risk factor for Neurosurgery patients.

Table 6

RISK FACTORS IN CANDIDURIA DUE TO *C. ALBICANS* AND *NON ALBICANS CANDIDA SPP.*

Risk factors, no of patients	Candiduria due to C.*albicans* n =14			Candiduria due to *nonalbicans Candida spp.* n=91		
	TOTAL no (%)	MALE no(%)	FEMALE, no (%)	TOTAL no(%)	MALE no (%)	FEMALE no(%)
Prolonged antibiotics,82	**11(78.6)**	6(43)	5(36)	**71(78)**	38(42)	33(36)
Catheterization,73	**6(42.9)**	3(21)	3(21)	**67(73.6)**	39(43)	28(31)
Diabetes mellitus,43	**6(42.9)**	4(29)	2(14)	**37(40.7)**	22(24)	15(17)
Chronic kidney disease,34	**3(21.4)**	1(7)	2(14)	**31(34)**	18(20)	13(14)
Unconsciousness,33	**2(14.3)**	-	2(14)	**31(34)**	14(15)	15(17)
Immunosuppressives, 27	**3(21.4)**	1(7)	2(14)	**24(26.4)**	13(14)	10(11)
Surgery,17	**2(14.3)**	2(14)	-	**15(16.5)**	9(10)	6(7)
Hemodialysis ,15	**2(14.3)**	-	2(14)	**13(14.3)**	9(10)	4(4)
Other Renal diseases,13	**2(14.3)**	-	2(14)	**11(12)**	2(2)	9(10)
ICU stay,12	**2(14.3)**	-	2(14)	**10(11)**	4(4)	6(7)
Renal transplant,8	**1(7.1)**	1(7)	-	**7(7.7)**	5(6)	2(2)
Other candidiasis (oral,esophageal),5	**-**	-	-	**3(3.3)**	3(3)	-
Use of antifungals,3	**-**	-	-	**3(3.3)**	3(3)	-
HIV reactive,2	**-**	-	-	**2(2.2)**	-	2(2)
Neurogenic bladder,2	**-**	-	-	**2(2.2)**	2(2)	-

*Other renal diseases- Acute Renal Failure, Tuberculosis of Kidney, RPGN-Rapidly Progressive Glomerulonephritis, Phimosis, Hypospadiasis, Calculi, Prostatomegaly, Phimosis, Carcinoma Prostate.

Major risk factors included antibiotics use in 82%, catheterization of urinary tract in 73%, followed by diabetes in 43% and Chronic Kidney Disease in 32% of the patients with Candiduria. *Nonalbicans Candida spp* was more common in catheterized patients (73.6%) and unconscious patients(34%).

Table 7

DISEASES OF URINARY TRACT IN PATIENTS WITH CANDIDURIA

Risk factor	Total, n=100 no (%)	Male, n=56 no (%)	Female, n=44 no(%)
Chronic kidney disease	34(34)	19(34)	15(34)
Acute renal failure	4(4)	1(1.8)	3(6.8)
Calculi	3(3)	2(3.6)	1(2.3)
Neurogenic bladder	2(2)	2(3.6)	-
Renal tuberculosis	1(1)	-	1(2.3)
RPGN	2(2)	-	2(4.5)
Prostatomegaly (BPH)	3(3)	3(5.4)	NA
CA Prostate	1(1)	1(1.8)	NA
Phimosis	1(1)	1(1.8)	NA
Hypospadiasis	1(1)	1(1.8)	NA

BPH-Benign Prostatic Hypertrophy, NA-Not applicable

Chronic Kidney Disease was the major risk factor among the diseases of urinary tract, accounting for about 34% of patients with Candiduria followed by Renal Transplantation in 8% of patients. Neurogenic bladder and Calculi were more common in males, while RPGN and TB were common in females.

Table 8

TYPE OF URINE SAMPLES

Type of sample Received	Total, n=100 no (%)	Male, n=56 no (%)	Female, n=44 no(%)
Catheterized urine	73(73)	42(75)	31(70.5)
Mid- stream urine	27(27)	14(25)	13(29.5%)
Suprapubic aspiration	0(0)	-	-
Total	100(100)	56(100)	44(100)

73 % of urine samples were catheterized urine samples followed by 27% of midstream urine samples. 75% of samples from males and 70.5% of samples from females were catheterized urine samples.

Table 9

DISTRIBUTION OF *CANDIDA ALBICANS* AND *NON-ALBICANS SPECIES* AMONG PATIENTS WITH CANDIDURIA

SPECIES	NO	PERCENTAGE
Candida albicans	14	13.3%
Nonalbicans Candida species	91	86.7%
Total	105	100%

Candida albicans accounted for only 13.3% of all *Candida spp* isolated, where as *nonalbicans Candida spp.* accounted for 86.7% among patients with Candiduria.

Table 10

DISTRIBUTION OF *CANDIDA SPECIES* AS SINGLE AND MIXTURE ISOLATE

Candida Species	**Single isolate, n=95 no (%)**	**Mixture isolates, n=10 no (%)**	**Total, n=105 ,no (%)**
C. tropicalis	64(67.4)	3(30)	67(63.8)
C. albicans	13(13.7)	1(10)	14(13.3)
C.guillermondii	8(8.4)	0(0)	8(7.6)
C.krusei	3(3.2)	5(50)	8(7.6)
C. parapsilosis	6(6.3)	0(0)	6(5.7)
C. kefyr	1(1.1)	1(10)	2(1.9)
C. glabrata	-	-	-
Total	95(100)	10(100)	105 (100)

Of the 105 isolates, 67 belonged to *C.tropicalis*, 14 isolates were *C.albicans*, followed by 8 isolates of *C.guillermondii* & *C. krusei* each. Six isolates of *C. parapsilosis and* 2 isolates of *C. kefyr* were also isolated. *C.tropicalis* was predominant among the single isolates (67.4%), where as *C.krusei* (50%) was predominant among mixture isolates.

Table 11

DISTRIBUTION OF MIXTURE ISOLATES OF *CANDIDA SPECIES* AMONG PATIENTS WITH CANDIDURIA

Mixture species	**no**	**Percent**
C.krusei& C.tropicalis	3	60%
C. krusei & C.albicans	1	20%
C. krusei & C.kefyr	1	20%
TOTAL	n=5	100%

C. krusei with *C. tropicalis* were the predominant mixtures (60%) obtained. *C.krusei* was found in all the mixture isolates.

Table 12

DISTRIBUTION OF *CANDIDA* ISOLATES AMONG CATHETERIZED AND MID-STREAM URINE SAMPLES

Species & Total no of isolates(%)	**Total no of isolates, n=105 no (%)**	**No of isolates in Catheterized samples, n=76 no (%)**	**No of isolates in Mid-stream urine, n=29 no (%)**
C.albicans	14(13.3)	5(6.6)	9(31.3)
C.tropicalis	67(63.8)	52(68.4)	15(51.7)
C. guillermondii	8(7.6)	6(7.8)	2(6.9)
C. krusei	8(7.6)	6(7.8)	2(6.9)
C. parapsilosis	6(5.7)	5(6.6)	1(3.4)
C. kefyr	2(1.9)	2(2.6)	0(0)
C. glabrata	-	-	-
Total	105(100%)	76(100%)	29(100%)

C.albicans was more common in mid stream urine (51.7%) sample than from catheterized urine samples. *C.tropicalis* was more common in catheterized urine samples (68.4%) than from midstream urine samples (51.7%).

Table 13

COMPARISON OF METHODS OF *CANDIDA* SPECIATION

SPECIES, NO OF ISOLATES	Sugar Assimilation, n(%)	Sugar Fermentation, n(%)	Hi-chrom agar, n(%)
*C.tropicalis,*67	67(100%)	67(100%)	58(86.5%)
C. albicans,14	14(100%)	13(92.9%)	13(92.8%)
C. guillermondii,8	8(100%)	8(100%)	7(87.5%)
C.krusei,8	8(100%)	8(100%)	8(100%)
C. parapsilosis,8	6(100%)	6(100%)	6(100%)
C.kefyr,2	2(100%)	2(100%)	0(0%)

Assimilation was the best method of *Candida* speciation as it identified all the *Candida* species (100%), followed by fermentation which identified all the species, except one isolate of *C.albicans.* The ability of Hi-Chrom agar to identify the species varied greatly from 86.5% to 100% in different species.

Table 14

SENSITIVITY OF HI-CHROM AGAR FOR VARIOUS SPECIES OF *CANDIDA*

Species, no of isolates	**Correctly identified by HI-Chrom agar**	**Wrongly Identified by HI-Chrom agar**	**Sensitivity**
*C.tropicalis,*67	58	9	86.5%
*C.albicans,*14	13	1	92.8%
*C.guillermondii,*8	7	1	87.5%
*C.krusei,*8	8	0	100%
*C.parapsilosis,*6	6	0	100%
*C.kefyr,*2	0	2	0%
C. glabrata,0	-	-	-
Total=105	92	13	87.6%

Hi media-Chrom agar was highly sensitive for detection of *C.parapsilosis* (100%) and *C.krusei* (100%), less sensitive for *C.tropicalis* (86.5%) & *C.guillermondii* (87.5%). Of the 105 isolates, only 92 were identified, with an overall sensitivity of 87.6%.

Table 15

SPECIFICITY, PREDICTIVE VALUES OF THE COLOURS ON HI-CHROM AGAR

SPECIES,COLOUR OF COLONIES & (n)	COLOURS ON Hi-CHROM AGAR							SPECIFICITY	PPV	NPV
	LG	DG	BL	CR	DP	PP-PU	PUR			
Steel blue ,*C.tropicalis* (67)	7	2	58	0	0	0	0	97.4%	98.3%	80%
Light green ,*C. albicans* (14)	13	0	1	0	0	0	0	92.8%	65%	98.9%
Pale pink to purple colonies, *C.guillermondii* (8)	0	1	0	0	0	7	0	100%	100%	99%
Dry pink, *C.krusei*, (8)	0	0	0	0	8	0	0	100%	100%	100%
Cream, *C.parapsilosis* (6)	0	0	0	6	0	0	0	100%	100%	100%
Purple, *C.glabrata,* (0)	-	-	-	-	-	-	-	-	-	-

Colonies- LG-Light Green, DG-Dark Green, BL-steel blue, CR –Cream, DP-dry,pink, PP- PU-pale pink to purple, PUR-purple,PPV-Positive predictive value, NPV-Negative predicitive value

Chrom agar was highly specific for *C.guillermondii ,C.parapsilosis* and *C.krusei* (100%) than those of *C.albicans* (92.8%) and *C.tropicalis* (97.4%).

Table 16

ANTIFUNGAL SUSCEPTIBILITY TO FLUCONAZOLE

SPECIES AND NUMBER OF ISOLATES(n)	SUSCEPTIBLE n(%) < 8 µg/ml		SUSCEPTIBLE DOSE DEPENDENT n(%),16-32 µg/ml		RESISTANT n(%) >64 µg/ml	
	DD	MBD	DD	MBD	DD	MBD
C.tropicalis(67)	46(68.7%)	48(71.6%)	6(8.9%)	4(5.9%)	15(22.4%)	15(22.4%)
C. albicans(14)	10(71.4%)	12(85.7%)	2(14.3%)	-	2(14.3%)	2(14.3%)
C.guillermondii(8)	7(87.5%)	7(87.5%)	-	-	1(12.5%)	1(12.5%)
C.krusei(8)	-	-		-	8(100%)	8(100%)
C.parapsilosis(6)	5(83.3%)	5(83.3%)	1(16.6%)	1(16.6%)	-	-
C.kefyr(2)	2(100%)	2(100%)	-	-	-	-
C. glabrata(0)	-	-	-	-	-	-
TOTAL(105)	70(67%)	74(70.5%)	9(8.6%)	5(4.7%)	26(24.7%)	26(24.7%)

DD-Disk Diffusion method, MBD-Microbroth Dilution technique

By Disk Diffusion, 70(67%) isolates were sensitive and 26(24.7%) isolates were resistant to Fluconazole, whereas by Microbroth Dilution technique 74(70.5%) isolates were sensitive and 26(24.7%) were resistant.

Table 17

ANTIFUNGAL SUSCEPTIBILITY TO ITRACONAZOLE

SPECIES AND NUMBER OF ISOLATES (n)	SUSCEPTIBLE n(%) < 0.125 µg/ml		SUSCEPTIBLE DOSE DEPENDENT n(%)0.25-0.5 µg/ml		RESISTANT n(%) >1 µg/ml	
	DD	MBD	DD	MBD	DD	MBD
C.tropicalis(67)	48(71.6%)	53(79.1%)	5(7.4%)	6(8.9%)	14(20.9%)	8(11.9%)
C. albicans(14)	8(57.1%)	10(71.4%)	2(14.2%)	2(14.2%)	4(28.6%)	2(14.2%)
C. guillermondii (8)	7(87.5%)	7(87.5%)	-	-	1(12.5%)	1(12.5%)
C.krusei(8)	5(62.5%)	5(62.5%)	-	1(12.5%)	3(32.5%)	2(25%)
C. parapsilosis (6)	6(100%)	6(100%)	-	-	-	-
C.kefyr(2)	2(100%)	2(100%)	-	-	-	-
C. glabrata(0)	-	-	-	-	-	-
TOTAL(105)	76(72.4%)	83(79.1%)	7(6.7%)	9(8.6%)	32(30.5%)	13(12.4%)

DD-Disk Diffusion method, MBD-Microbroth Dilution technique

79.1% of the *Candida* isolates were sensitive, 8.6% were susceptible dose dependent and 12.4% were resistant to Itraconazole by Microbroth Dilution technique.

Table 18

ANTIFUNGAL SUSCEPTIBILITY TO AMPHOTERICIN B

SPECIES AND NO OF ISOLATES	SUSCEPTIBLE n(%) < 1µg/ml		RESISTANT n(%) >1 µg/ml	
	DD	MBD	DD	MBD
C.tropicalis,67	61(91.7%)	67(100%)	6(8.9%)	-
C. albicans,14	12(85.7%)	14(100%)	2(14.2%)	-
C .guillermondii,8	6(75%)	8(100%)	2(25%)	-
C.krusei,8	5(62.5%)	8(100%)	3(37.5%)	-
C. .parapsilosis,6	5(83.3%)	6 (100%)	1(16.67%)	-
C.kefyr,2	2(100%)	2(100%)	-	-
*C.glabrata,*0	-	-	-	-
TOTAL(105)	91(86.7%)	105(100%)	14(13.3%)	-

DD-Disk Diffusion method, MBD-Microbroth Dilution technique.

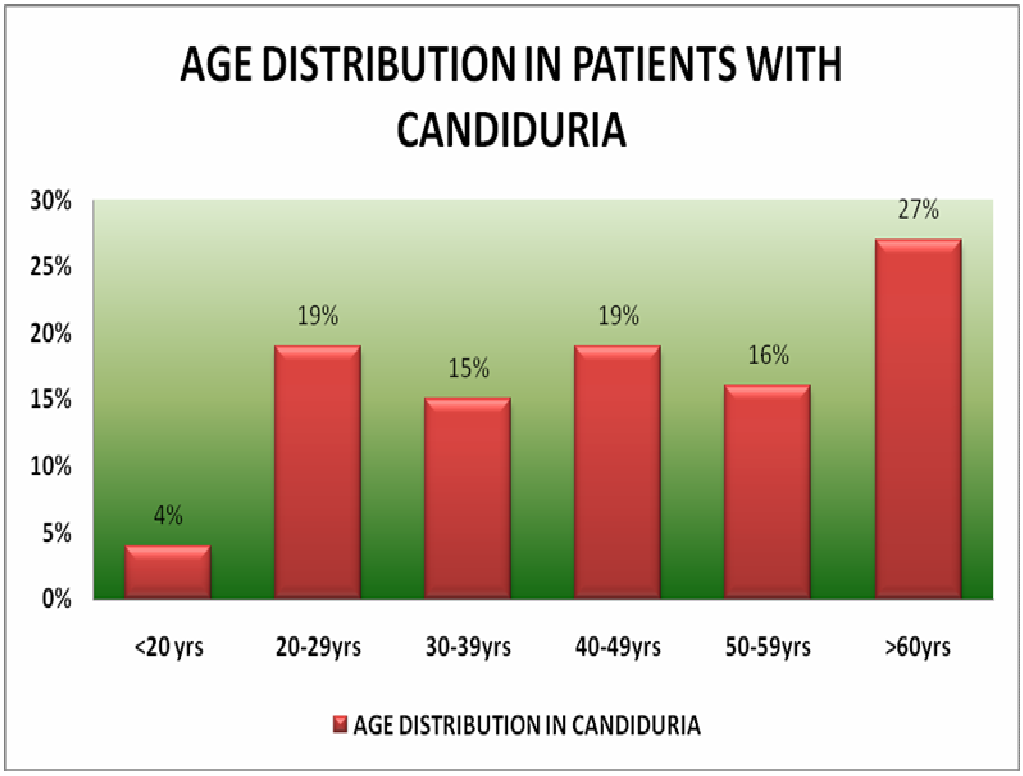
All the isolates were highly sensitive (100%) to Amphotericin B by MBD.

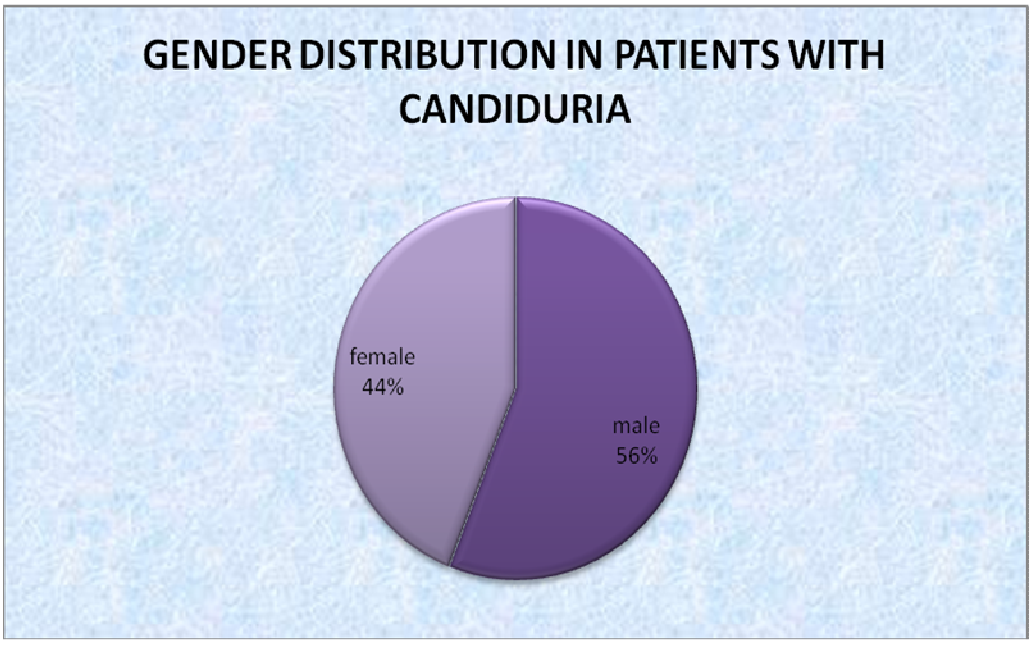
Table 19

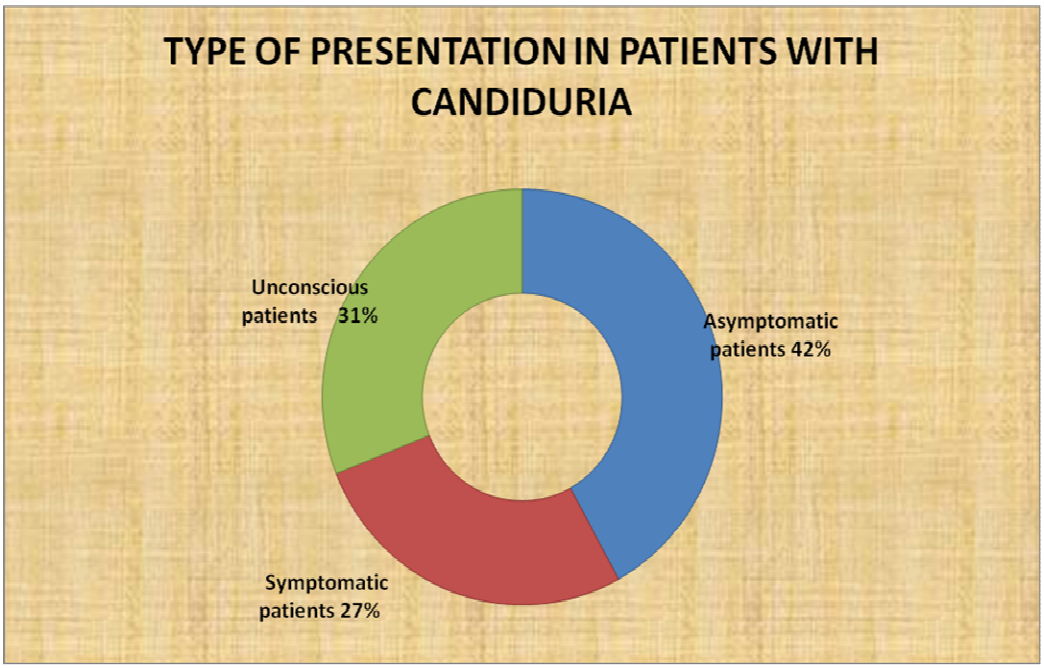
DISCREPANCIES IN DISK DIFFUSION & MICROBROTH DILUTION

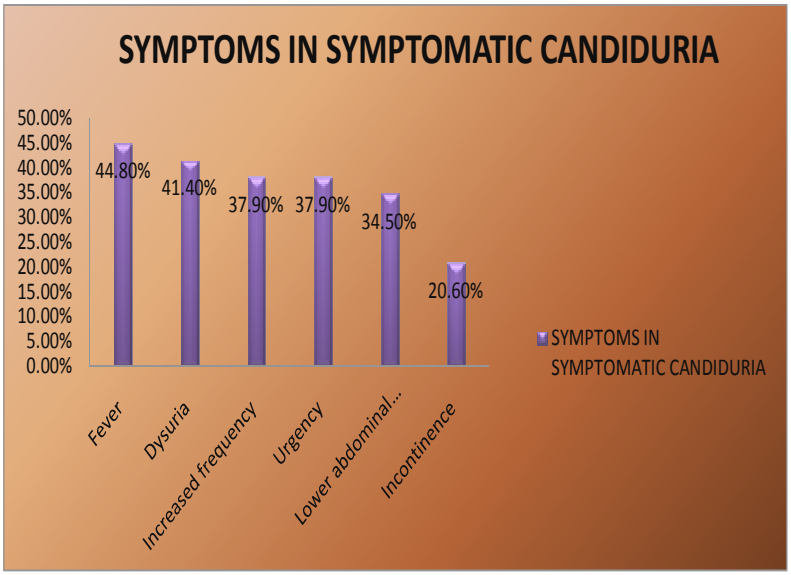
Antifungal	Minor error	Major error	Very major error
Fluconazole	4 (3.8%)	-	-
Itraconazole	12(11.4%)	2(1.9%)	-
Amphotericin B	-	14(13.3%)	-

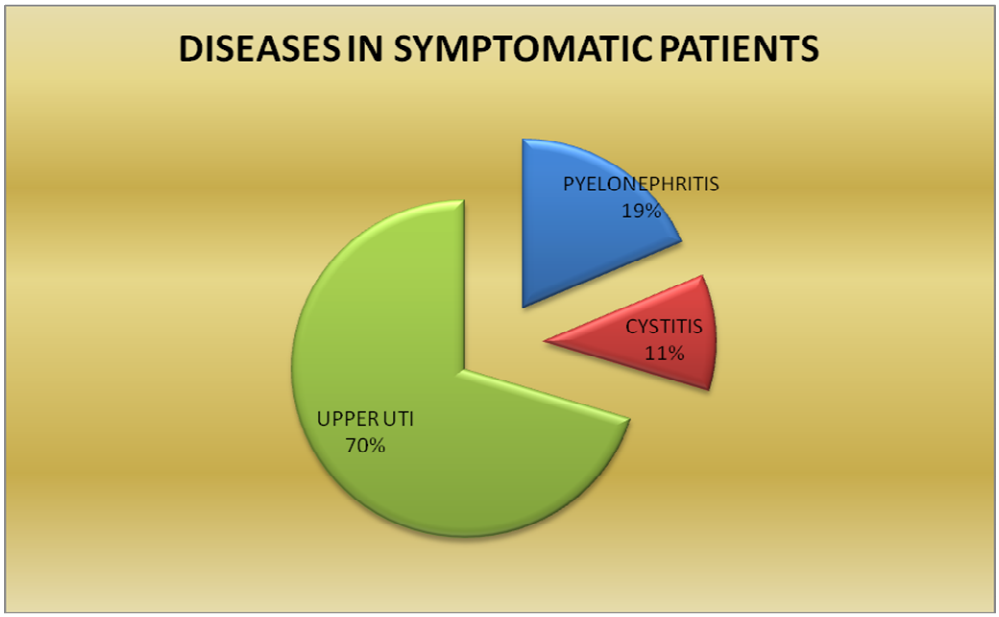
- Minor error-Shifts between Susceptible and SDD or Resistant and SDD by DD and MBD.
- Major error-Resistant by DD but Susceptible by MBD.
- Very Major Error- Sensitive by DD but Resistant by MBD.

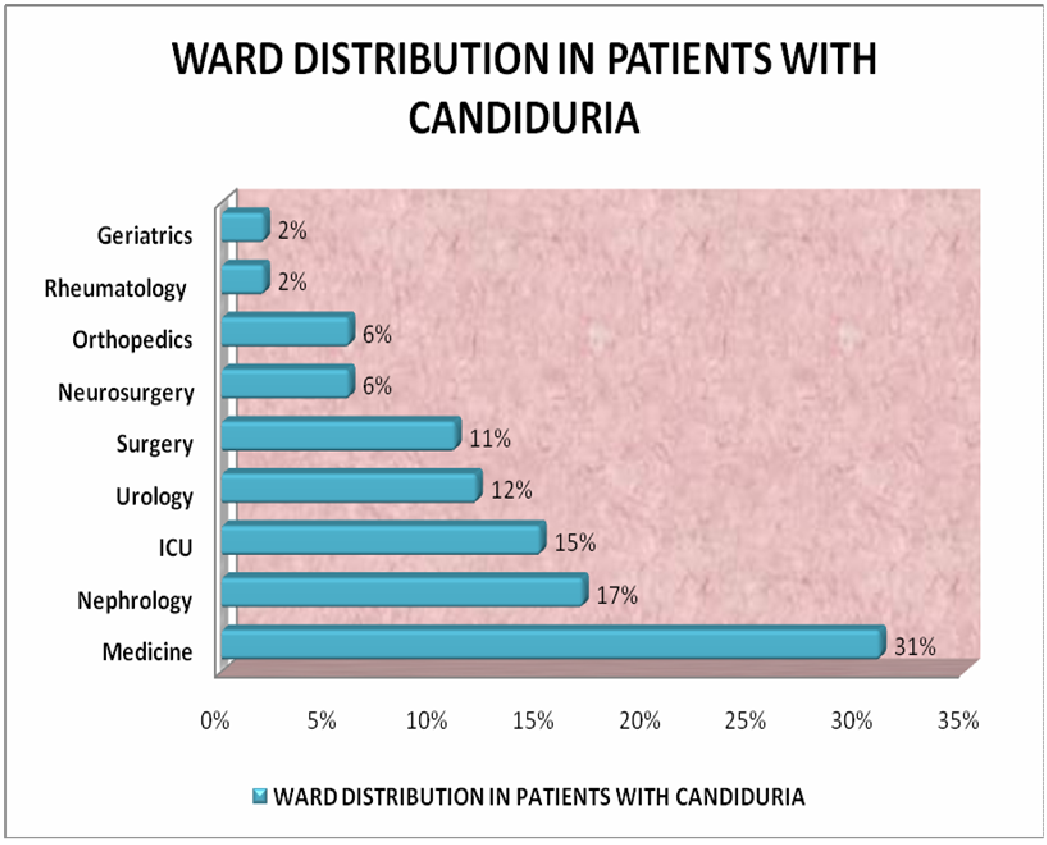

AGE DISTRIBUTION IN PATIENTS WITH CANDIDURIA
30%
25%
20%
15%
10%
5%
0%
4%
19%
15%
19%
16%
27%
<20 yrs
20-29yrs
30-39yrs
40-49yrs
50-59yrs
>60yrs
AGE DISTRIBUTION IN CANDIDURIA

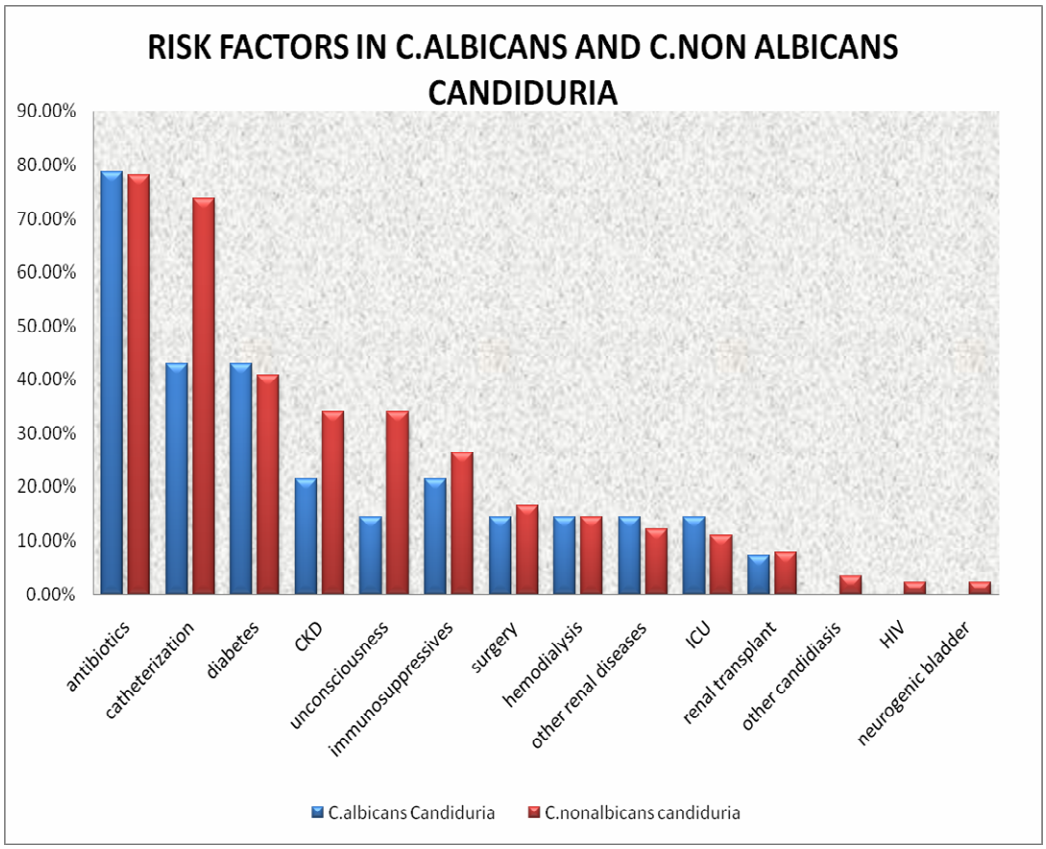

GENDER DISTRIBUTION IN PATIENTS WITH CANDIDURIA
female 44%
male 56%

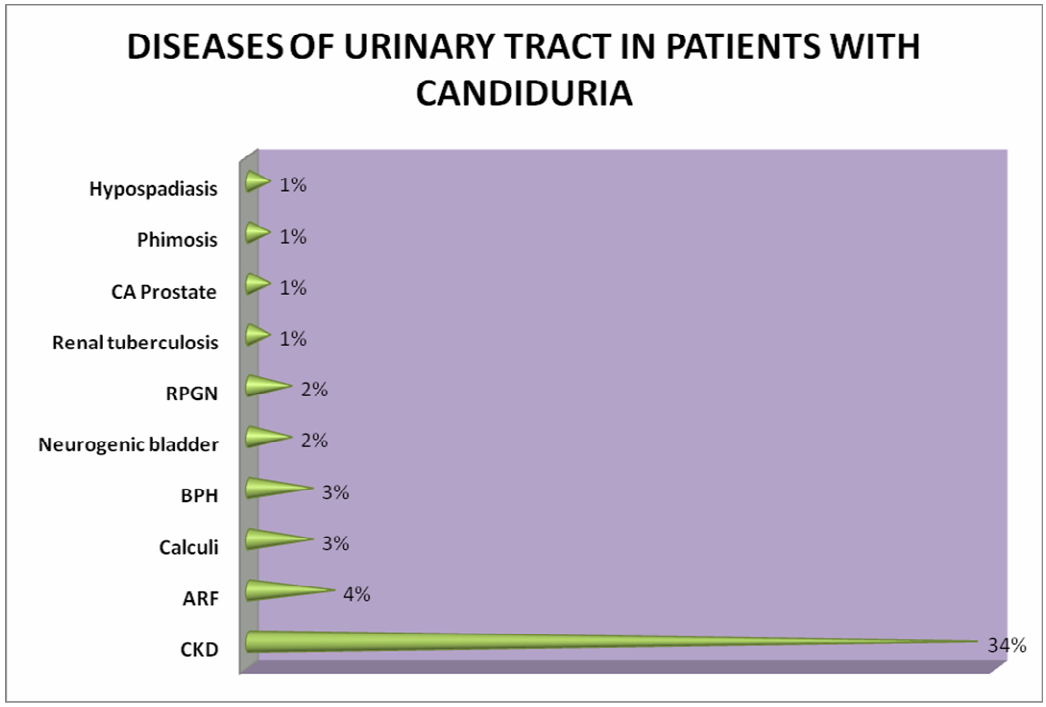

TYPE OF PRESENTATION IN PATIENTS WITH CANDIDURIA
Unconscious patients 31%
Asymptomatic patients 42%
Symptomatic patients 27%

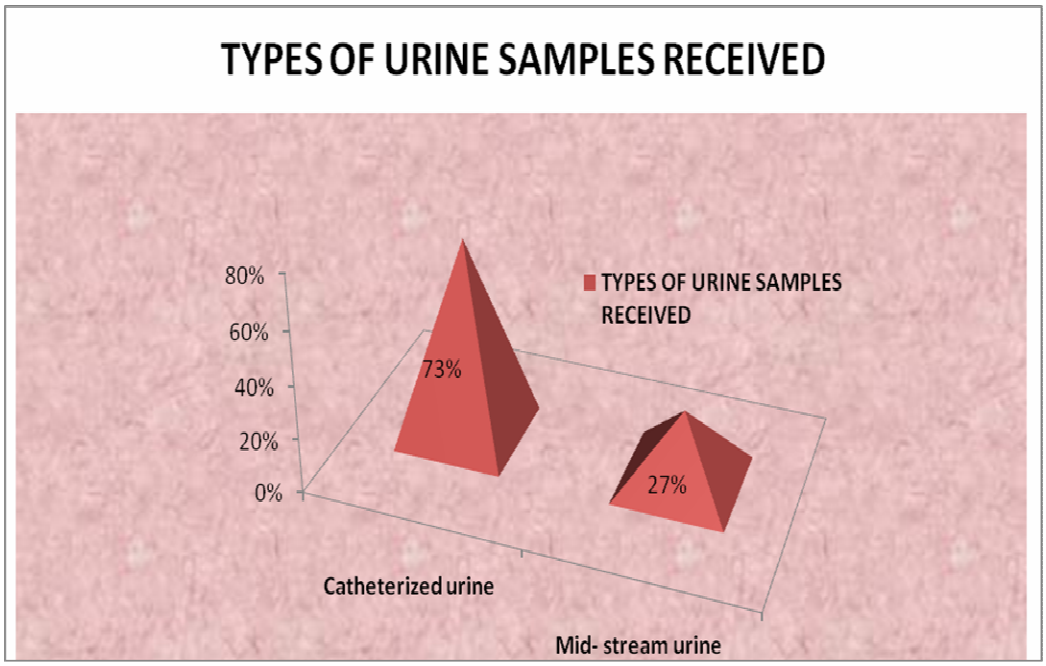

SYMPTOMS IN SYMPTOMATIC CANDIDURIA
50.00%
45.00%
40.00%
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
44.80%
41.40%
37.90%
37.90%
34.50%
20.60%
Fever
Dysuria
Increased frequency
Urgency
Lower abdominal...
Incontinence
SYMPTOMS IN SYMPTOMATIC CANDIDURIA

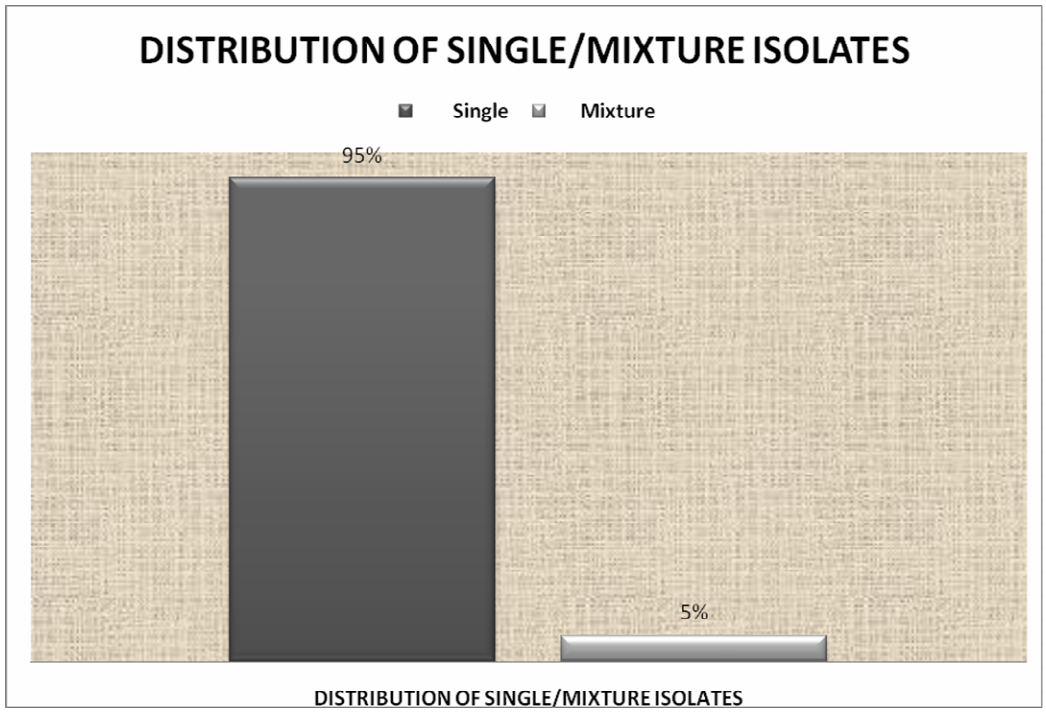

DISEASES IN SYMPTOMATIC PATIENTS
PYELONEPHRITIS
19%
CYSTITIS
11%
UPPER UTI
70%

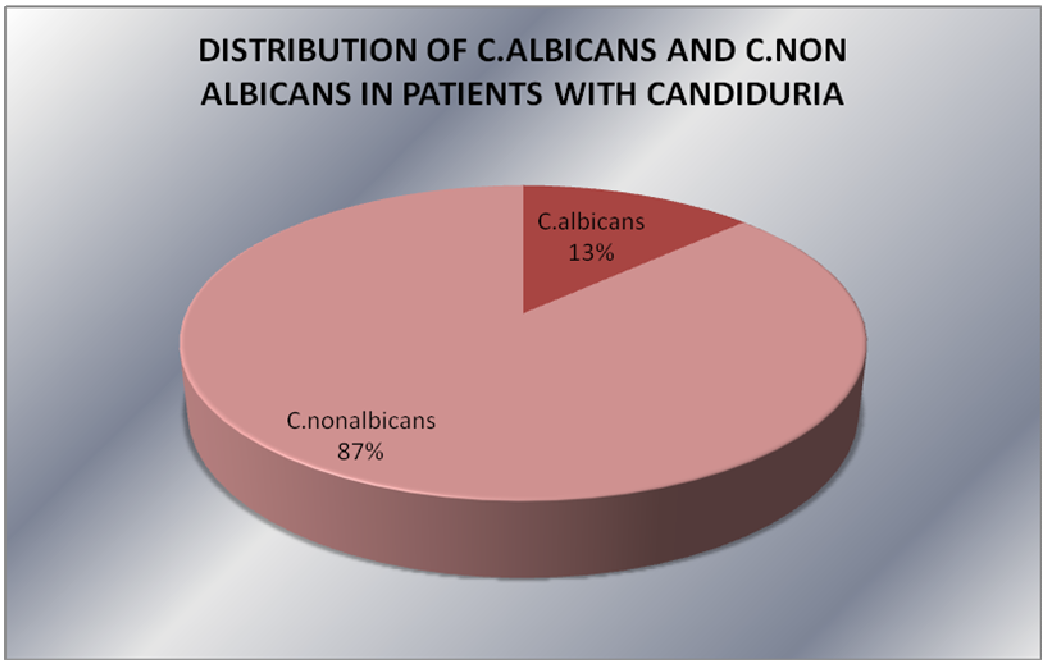

WARD DISTRIBUTION IN PATIENTS WITH CANDIDURIA
Geriatrics
Rheumatology
Orthopedics
Neurosurgery
Surgery
Urology
ICU
Nephrology
Medicine
2%
2%
6%
6%
11%
12%
15%
17%
31%
0%
5%
10%
15%
20%
25%
30%
35%
WARD DISTRIBUTION IN PATIENTS WITH CANDIDURIA

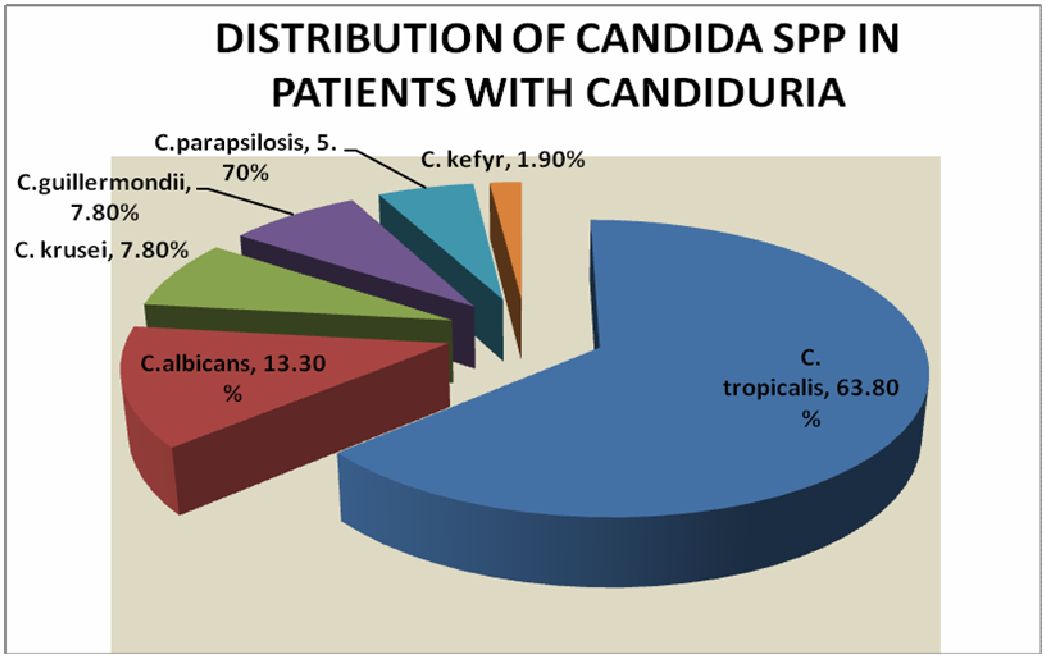

RISK FACTORS IN C.ALBICANS AND C.NON ALBICANS CANDIDURIA
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
antibiotics
catheterization
diabetes
CKD
unconsciousness
immunosuppressives
surgery
hemodialysis
other renal diseases
ICU
renal transplant
other candidiasis
HIV
neurogenic bladder
C.albicans Candiduria
C.nonalbicans candiduria

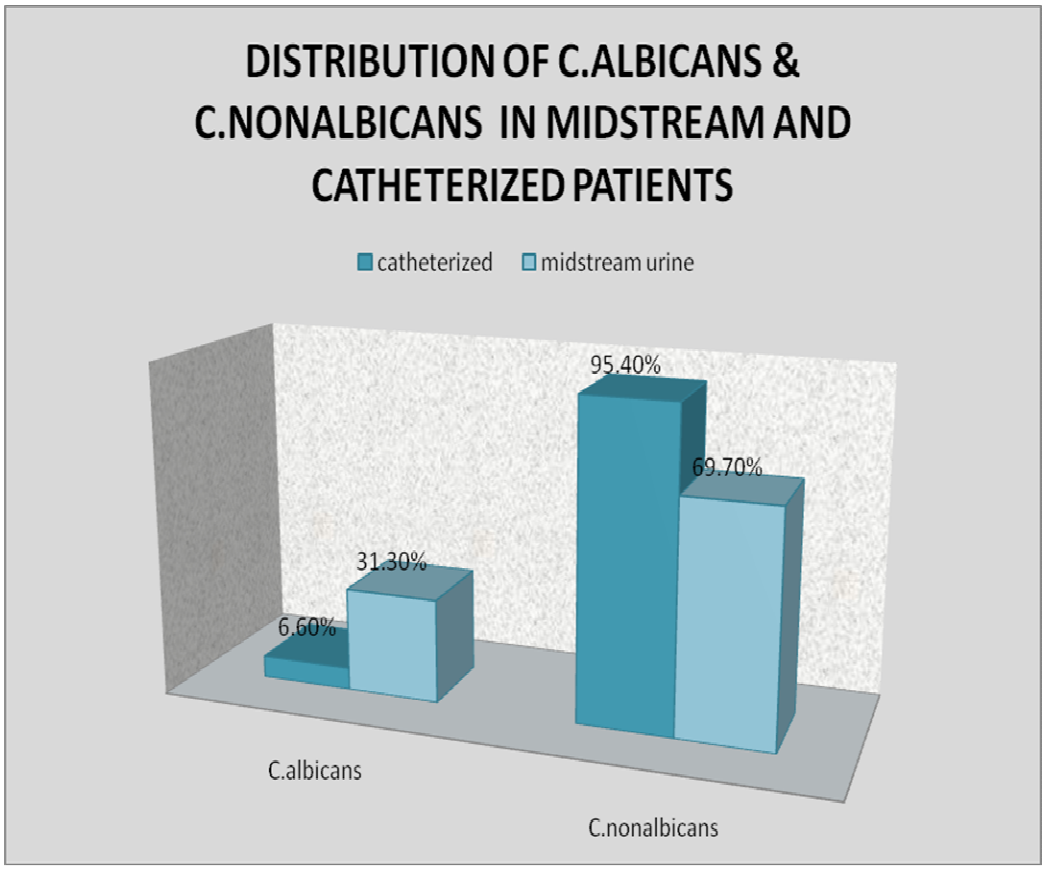

DISEASES OF URINARY TRACT IN PATIENTS WITH CANDIDURIA
Hypospadiasis
1%
Phimosis
1%
CA Prostate
1%
Renal tuberculosis
1%
RPGN
2%
Neurogenic bladder
2%
BPH
3%
Calculi
3%
ARF
4%
CKD
34%

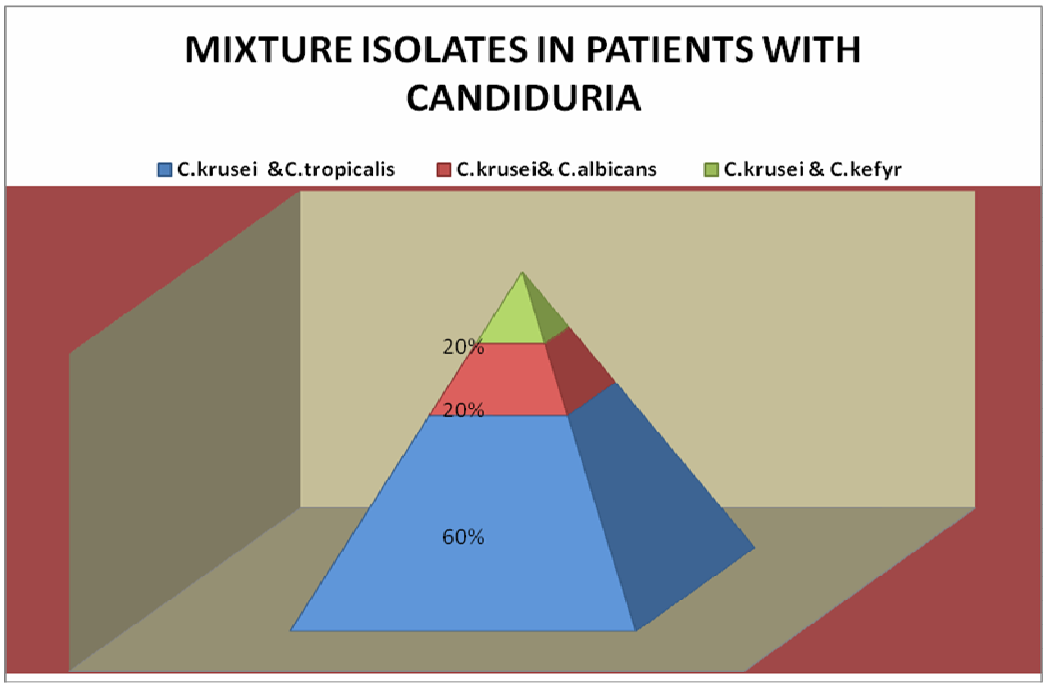

TYPES OF URINE SAMPLES RECEIVED
80%
60%
40%
20%
0%
73%
27%
TYPES OF URINE SAMPLES RECEIVED
Catheterized urine
Mid- stream urine

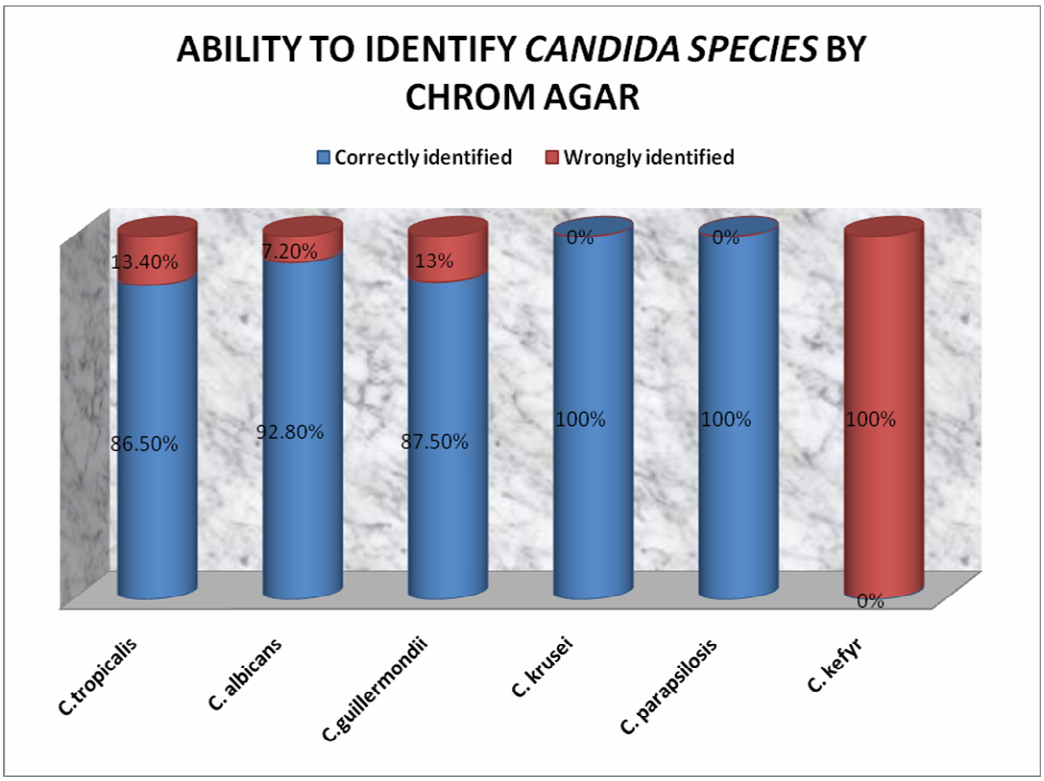

DISTRIBUTION OF SINGLE/MIXTURE ISOLATES
Single
Mixture
95%
5%
DISTRIBUTION OF SINGLE/MIXTURE ISOLATES

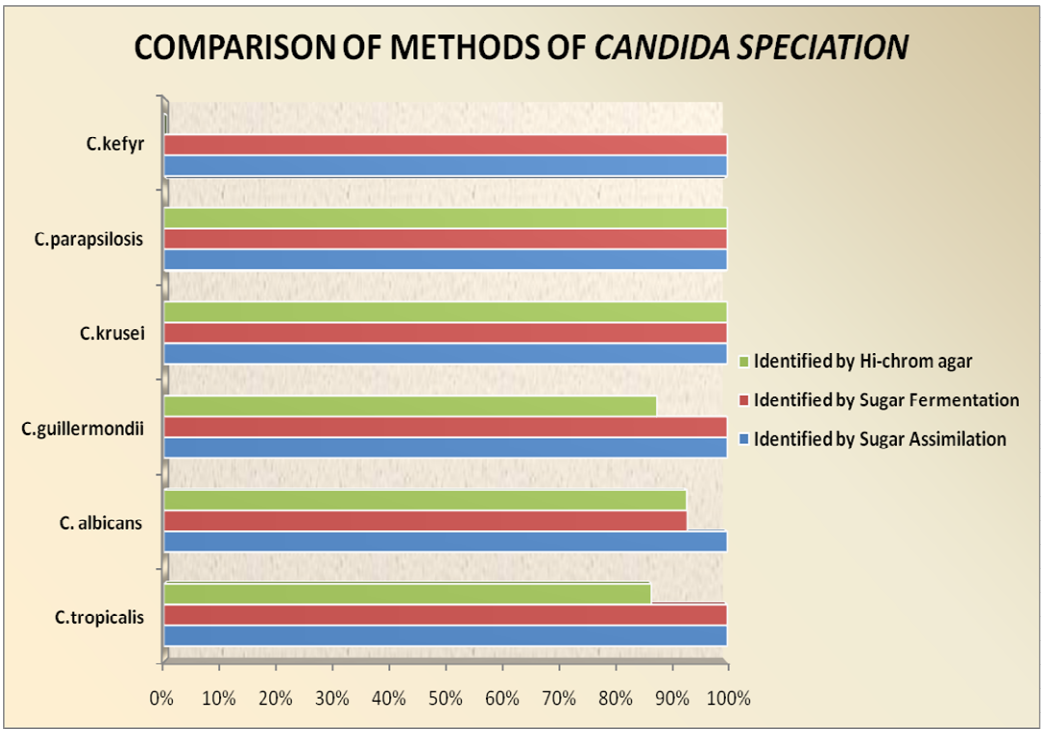

DISTRIBUTION OF C.ALBICANS AND C.NON ALBICANS IN PATIENTS WITH CANDIDURIA
C.albicans 13%
C.nonalbicans 87%


DISTRIBUTION OF CANDIDA SPP IN PATIENTS WITH CANDIDURIA
C.parapsilosis, 5. 70%
C. kefyr, 1.90%
C.guillermondii, 7.80%
C. krusei, 7.80%
C.albicans, 13.30 %
C. tropicalis, 63.80 %

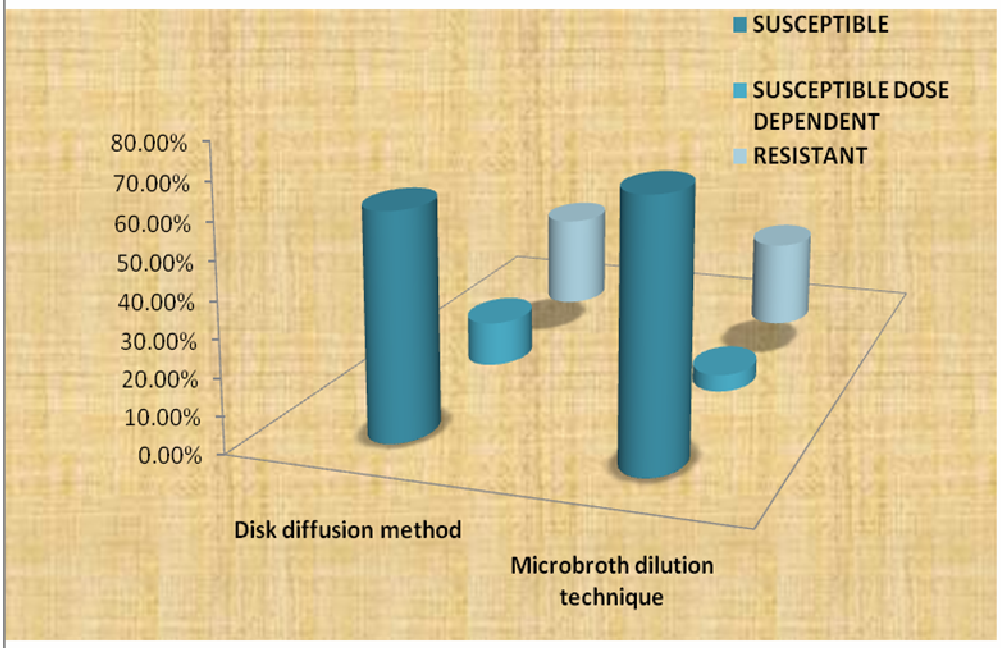

DISTRIBUTION OF C.ALBICANS & C.NONALBICANS IN MIDSTREAM AND CATHETERIZED PATIENTS
catheterized
midstream urine
95.40%
69.70%
31.30%
6.60%
C.albicans
C.nonalbicans


MIXTURE ISOLATES IN PATIENTS WITH CANDIDURIA
C.krusei &C.tropicalis
C.krusei& C.albicans
C.krusei & C.kefyr
20%
20%
60%

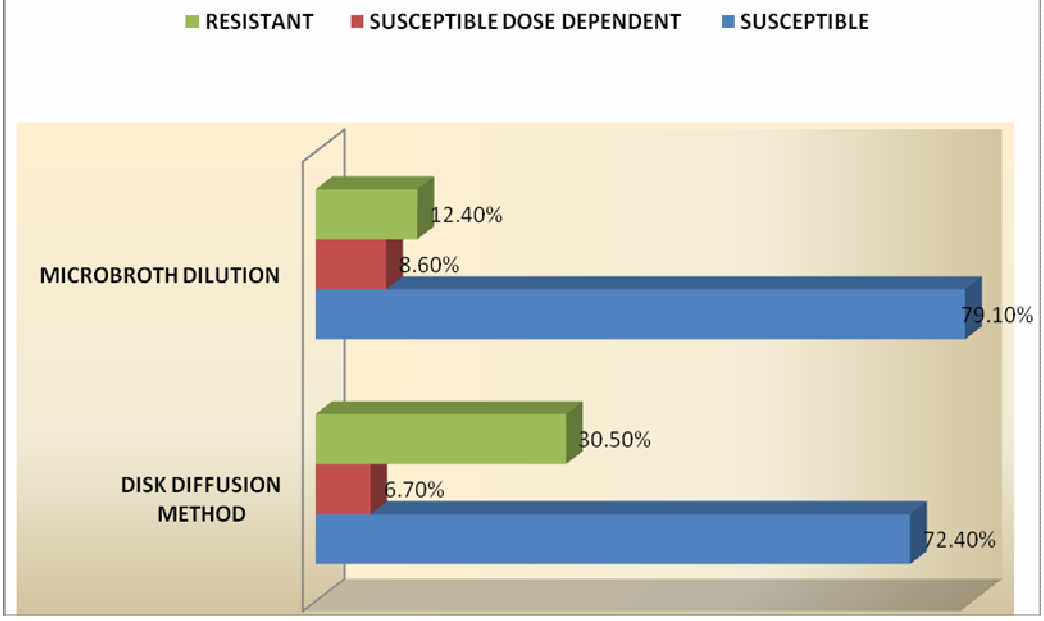

ABILITY TO IDENTIFY CANDIDA SPECIES BY CHROM AGAR
Correctly identified
Wrongly identified
13.40%
86.50%
7.20%
92.80%
13%
87.50%
0%
100%
0%
100%
100%
0%
C.tropicalis
C. albicans
C.guillermondii
C. krusei
C. parapsilosis
C. kefyr

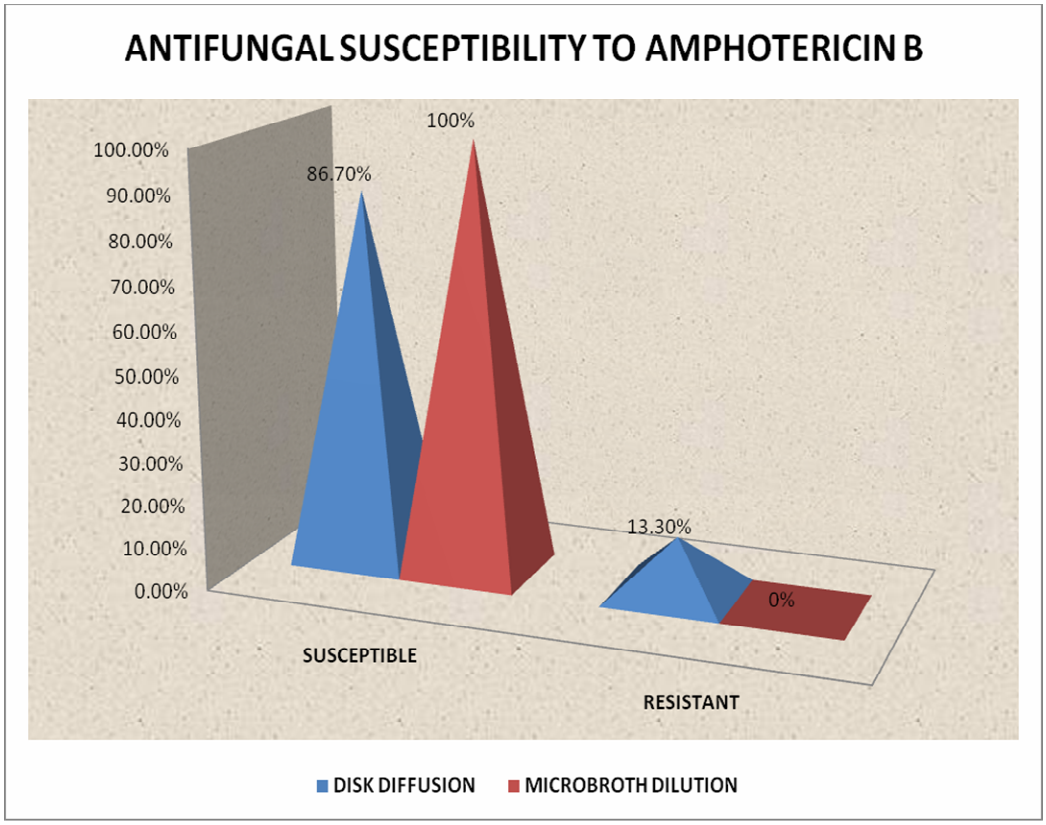

COMPARISON OF METHODS OF CANDIDA SPECIATION
C.kefyr
C.parapsilosis
C.krusei
C.guillermondii
C. albicans
C.tropicalis
Identified by Hi-chrom agar
Identified by Sugar Fermentation
Identified by Sugar Assimilation
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%

ANTIFUNGAL SUSCEPTIBILITY TO FLUCONAZOLE



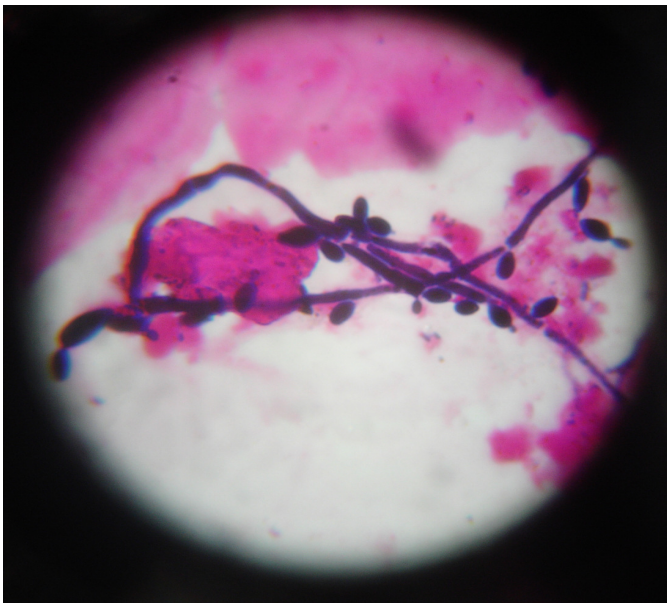
ANTIFUNGAL SUSCEPTIBILITY OF ITRACONAZOLE



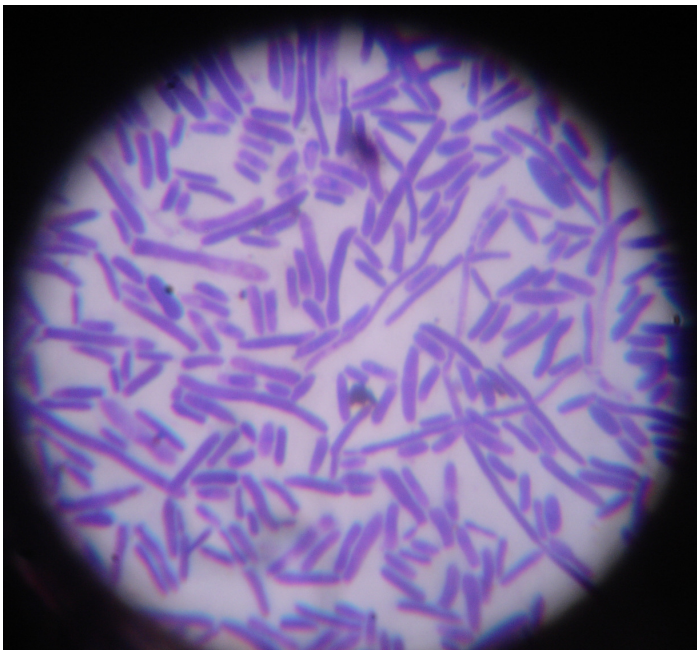

ANTIFUNGAL SUSCEPTIBILITY TO AMPHOTERICIN B
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
86.70%
100%
13.30%
0%
SUSCEPTIBLE
RESISTANT
DISK DIFFUSION
MICROBROTH DILUTION

CREAM COLOURED COLONIES OF *C.tropicalis* WITH MYCELIAL FRINGE

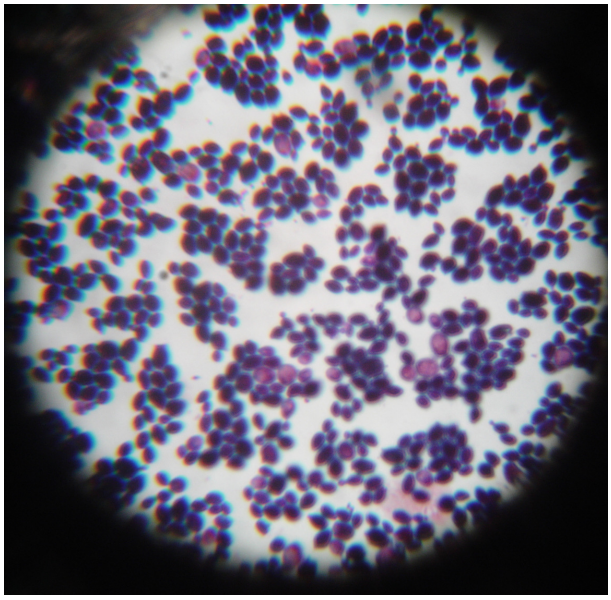
FLAT,DRY, WRINKLED COLONIES OF *C.krusei*



GRAM'S STAIN OF CENTRIFUGED URINE SHOWING PSEUDOHYPHAE OF *CANDIDA SPP.*



ELONGATED SLENDER BLASTOCONIDIA OF *C.krusei*

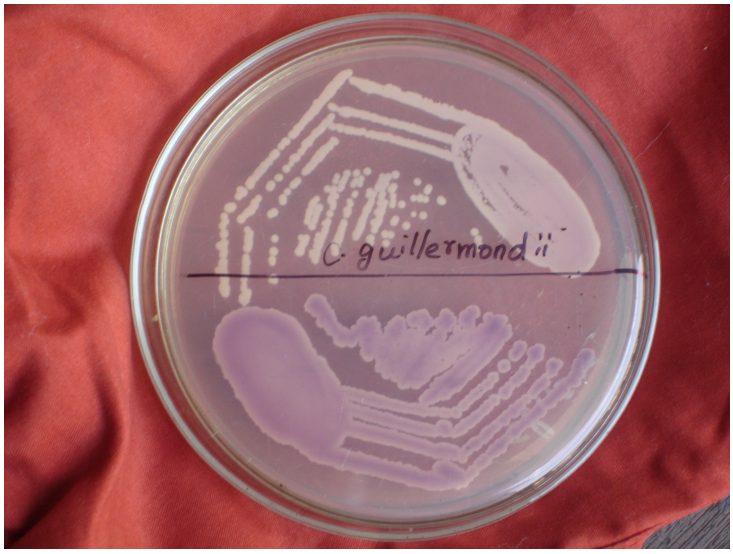


ROUND BLASTOCONIDIA OF *C.albicans*

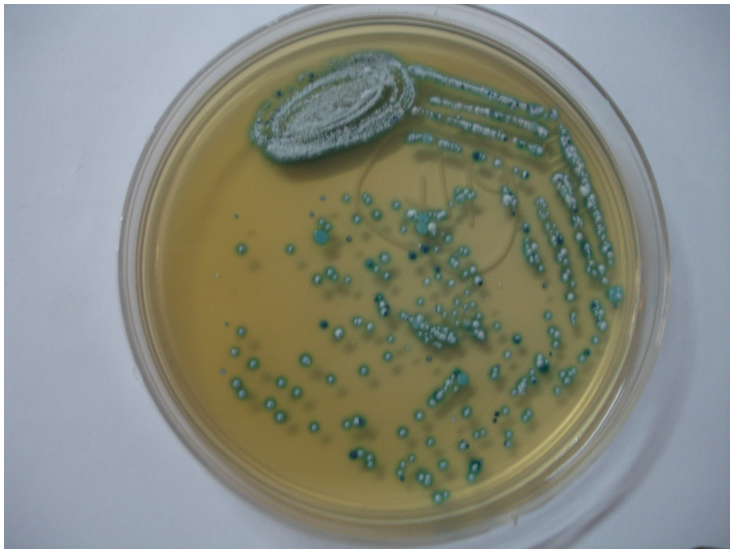
STEEL BLUE COLONIES OF *C.tropicalis* ON CHROM AGAR

LIGHT GREEN COLONIES OF *C.albicans* ON CHROM AGAR

CREAM COLOURED COLONIES OF *C.parapsilosis* ON CHROM AGAR



CREAM TO PALE PINK,PURPLE COLONIES OF *C.guillermondii* ON CHROM AGAR



SEPERATION OF MIXTURE *CANDIDA SPP.* ON CHROM AGAR

SUGAR FERMENTATION PATTERN OF *C.tropicalis*

SUGAR FERMENTATION PATTERN OF *C.parapsilosis*

SUGAR FERMENTATION OF *C.krusei*

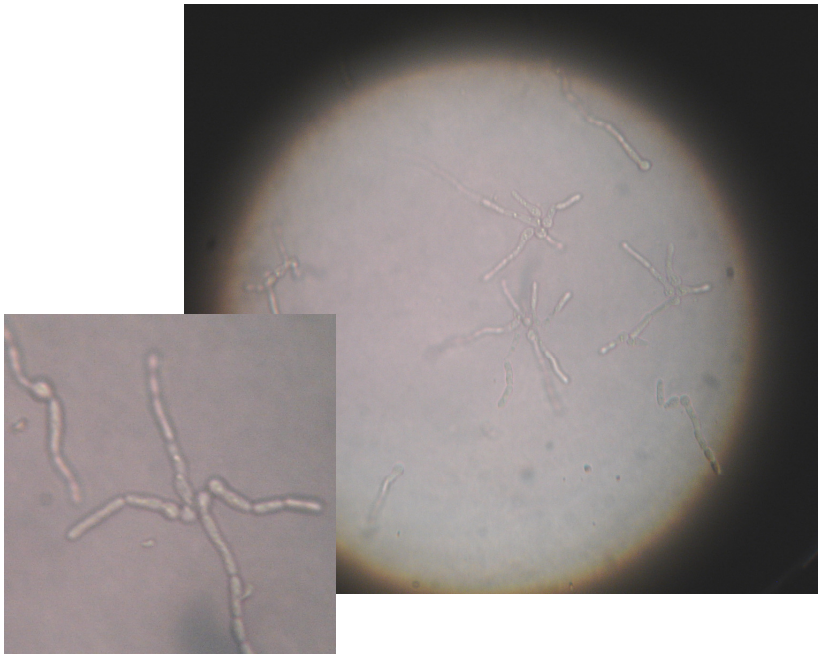
SUGAR FERMENTATION OF *C.albicans*

SUGAR FERMENTATION PATTERN OF *C.guillermondii*

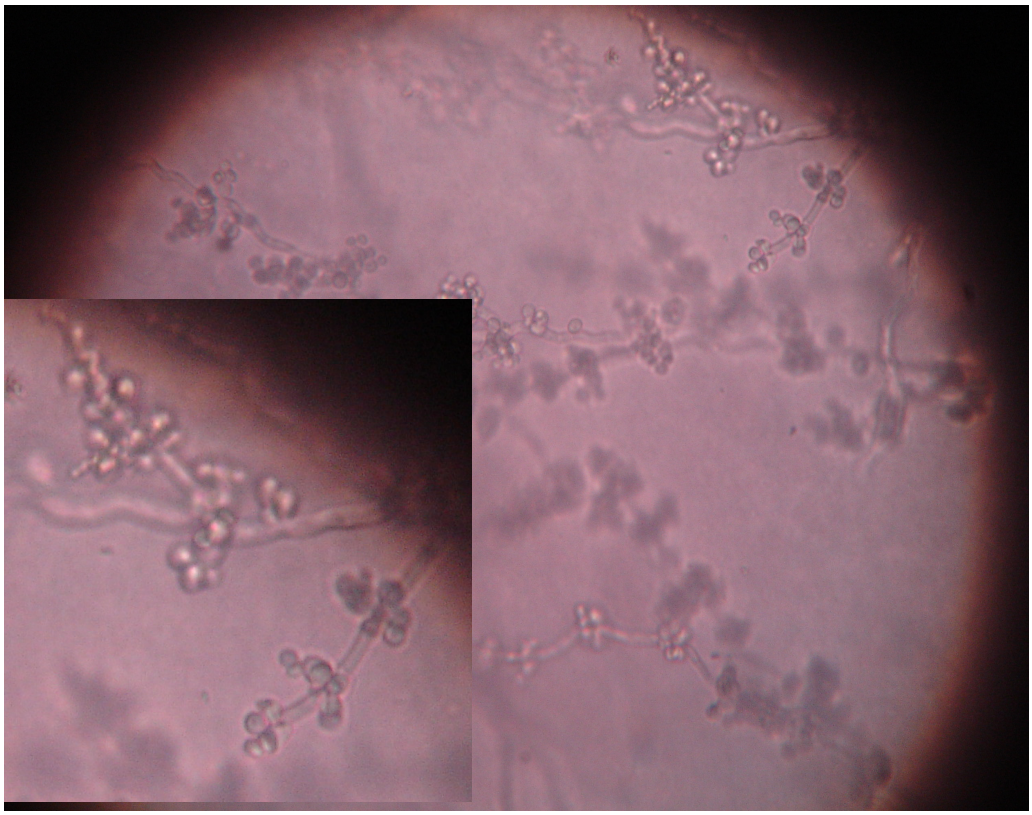
***C.tropicalis* ON CMA-OVAL BLASTOCONIDIA ANYWHERE**

ALONG THE PSEUDOHYPHAE

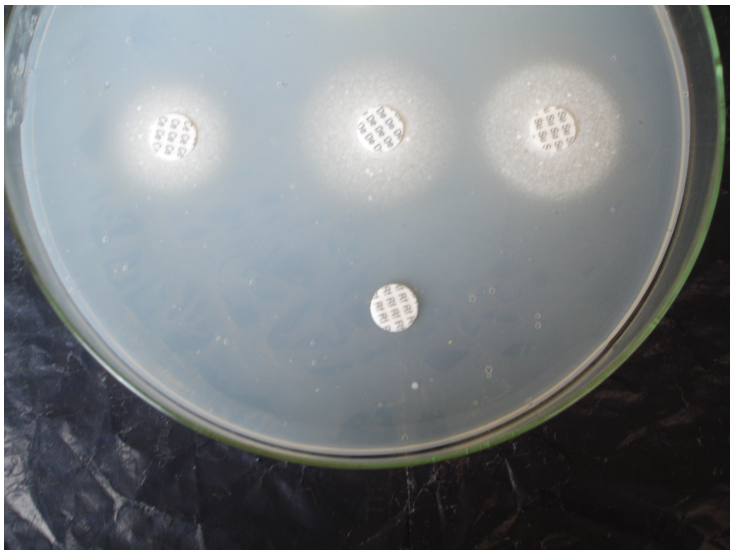
C.guillermondii **ON CMA- BLASTOCONIDIA AT CONSTRICTIONS OF PSEUDOHYPHAE**



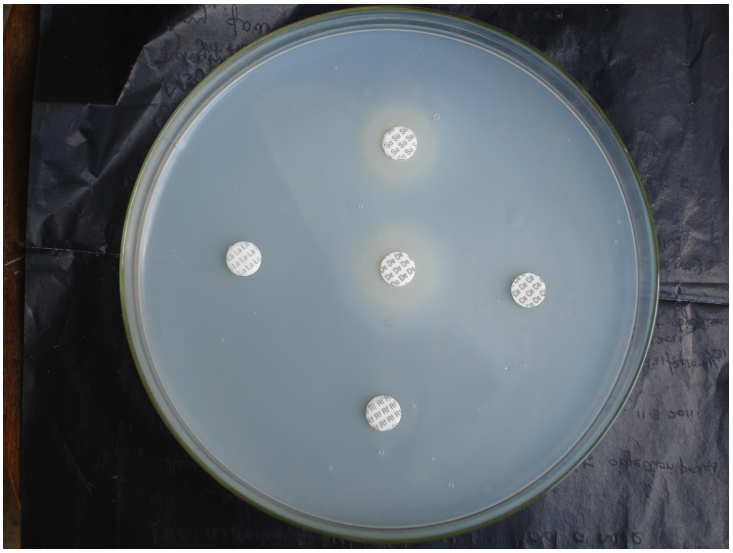
CROSSED MATCH STICK APPEARANCE OF *C.krusei*



CHLAMYDOSPORES OF *C.albicans*

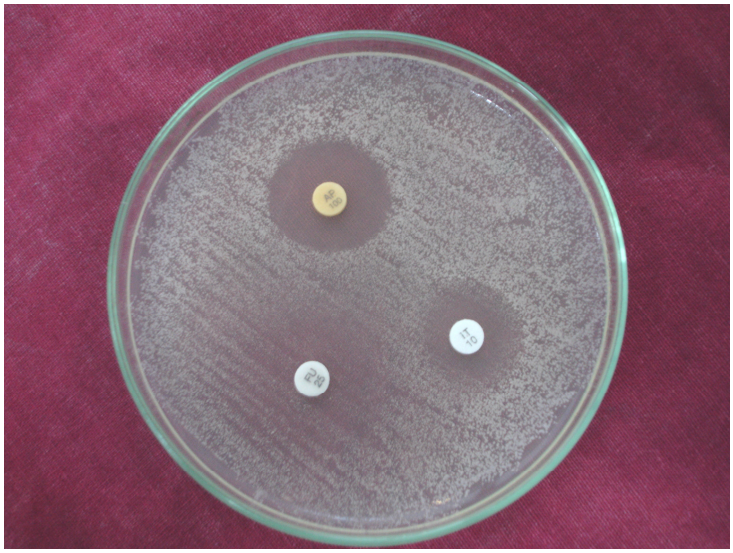


SUGAR ASSIMILATION PATTERN OF *C.tropicalis*

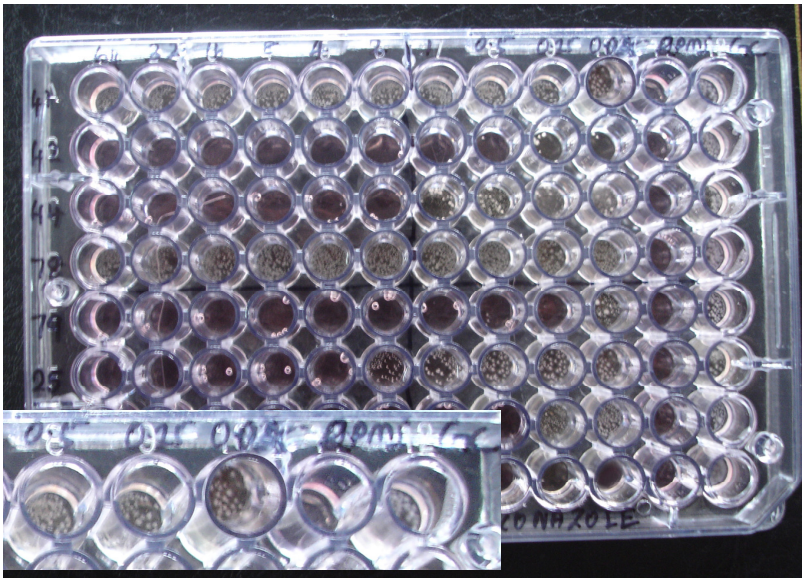


SUGAR ASSIMILATION PATTERN OF *C.albicans*

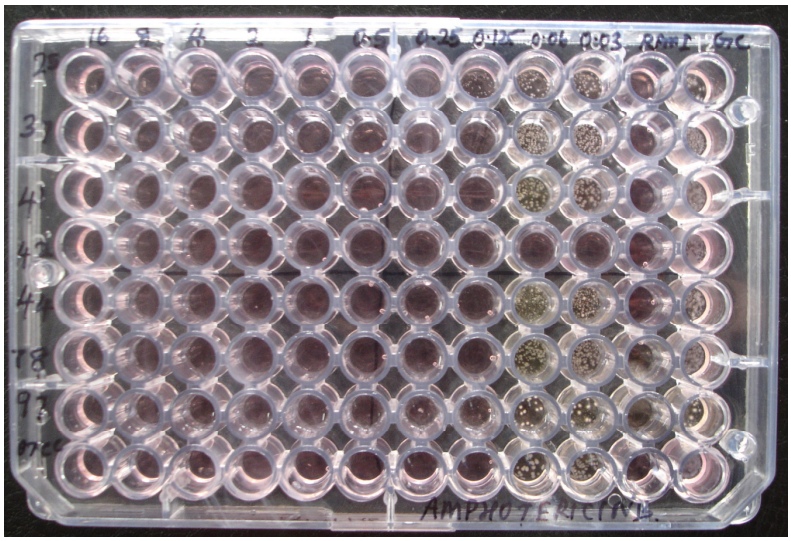
ANTIFUNGAL SUSCEPTIBILITY BY DISK DIFFUSION METHOD



FLUCONAZOLE RESISTANT ISOLATE IN DISK DIFFUSION METHOD



MICROBROTH DILUTION TECHNIQUE FOR FLUCONAZOLE



MICROBROTH DILUTION TECHNIQUE FOR AMPHOTERICIN B

DISCUSSION

A significant rise in prevalence of Urinary Tract Infections(UTI) due to *Candida spp.* has occurred over the last 20 years with the surge of *non albicans Candida spp*. Clinical importance of species level identification is important as they differ in expression of virulence factors and Antifungal susceptibility.

Current study was undertaken to speciate the *Candida* isolated from urine samples of hospitalized patients and to find their Antifungal susceptibility pattern. The study also concentrated on the changes observed in species distribution, the shift towards *nonalbicans Candida spp.* in our hospital.

The present study showed predominance of patients in the age group of >60 yrs (Table1) contributing to about 27% of total patients, followed by patients in 19% in 40-49 yrs and 20-29 yrs. This correlated well with the previous study by S.Krcmery et al, were the mean age was 62.4 yrs[5].

Males contributed to 56% (Table1) of the study population. This was similar to the results of study by Arlene O.Cantillep et al [87]. Although there is an increased risk in female gender, the other associated risk factors like Diabetes, CKD, Prostatomegaly, Carcinoma prostate were common in males in our study.

Female population predominated in 20-29 yrs and 30-39 yrs, probably because of increased vaginal colonization in the sexually active population, use of contraceptive devices , contraceptive pills and poor genital hygiene in low socioeconomic group. High levels of reproductive hormones provide a greater

amount of glycogen in the vagina, further providing a good source of carbon for *Candida* growth. It was demonstrated that estrogens increases the affinity for the vaginal epithelial cell adherence of *Candida* and Yeast cytosol receptor or system to connect to reproductive hormones that increase the formation of yeast blastopores[88].

An important observation was that about 42% (Table 2) of patients with Candiduria were asymptomatic; an important complicating factor in defining Candiduria, as many patients on long term catheterization cannot vocalize on symptoms of dysuria or increased frequency. Asymptomatic patients were common in the age groups 40-49 and 50-59 yrs. Of the 42 patients with asymptomatic Candiduria, 30 were on antibiotics and 29 patients were catheterized.

Only 13% of patients in one large multicenter study by Mauricio Carvalho et al in 2001, had symptoms suggesting UTI. Current study showed the symptomatic Candiduria in 27% (Table 2) of patients. Symptoms of UTI were predominant in the age group of > 60 yrs and were more common in males. The predominance was probably because of increase in associated risk factors in the older age group.

In the present study, about 31% (Table 2) of patients were unconscious and on prolonged catheterization. So these patients could not be categorized either as symptomatic or asymptomatic patients. About 10 of the 12 ICU patients, 5 of 6 neuro-surgery patients and 10 patients from medical ward were unconscious. These patients had associated risk factors like catheterization and prolonged antibiotic use.

Fever was the most common presenting symptom in 16 % of patients with Candiduria followed by dysuria in 11% (Table 3). This was lesser than the results of the study by Paul A Tambyah et al[89], where in fever was present in 17.7% patients and dysuria in 6% of catheterized patients with UTI . Tambyah et al's study population was that of both bacterial and fungal infections in catheterized patients, were as the current study included only patients with Candiduria. Catheterization also makes the patient asymptomatic, as the presence of a catheter in the urethra prevents continuous exposure of urethral mucosa to organisms in infected urine, preventing infectious urethritis that produces dysuria, urgency in infected noncatheterized patients. A patent urinary catheter ensures that urinary tract is continuously decompressed, preventing urgency and frequency associated with distension of an inflamed bladder and Vesicoureteral reflux [89]. Five of these patients had Pyelonephritis, 3 patients had Cystitis, and 19 patients had lower UTI(Table 4).

Most of the patients in our study belonged to Medical ward, contributing to 35% of patients, followed by Nephrology (17%) (Table 5). This was similar to the study by Stephen P storfer [90].The risk factors were also found to vary in different wards. Diabetes mellitus was the most common risk factor in patients from medical wards, CKD and other diseases of urinary tract were common in Nephrology and Urology. ICU and surgery ward patients showed antibiotics and catheterization as the common risk factors [87].

Antibiotic use (82%) and catheterization (73%) were the most common risk factor associated (Table 6). This was lower than the results by Uma Chaudary et al [91] ,who showed antibiotics as a risk factor in 99.6% of patients,

catheterization in 90%, because the study population in her study included Critically Ill patients with Candiduria rather than the hospitalized patients in our study. Antibiotics alter the normal flora of the genito-urinary tract, thus paving way for colonization by *Candida species*. The surface of catheters also aid in colonization with *non albicans Candida spp* .There was no statistically significant association between *nonalbicans Candida spp* and antibiotic use, with a 'p' value of 0.792.

Diabetes mellitus (43%) was most common disease associated, followed by CKD (34%). This was slightly higher than the study by Cl´audia Castelo Branco et al[93], who showed 26.7% of the patients, had diabetes probably because; Indians are more prone to diabetes. India is termed as the "diabetes capital of the world". According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 due to "Asian Indian Phenotype" refers to certain unique clinical and biochemical abnormalities in Indians which includes increased insulin resistance, greater abdominal adiposity, lower adiponectin and higher C-Reactive Protein levels. [92]

Immuno suppressives like steroids (oral and injectable), tacrolimus and mycophenolate mofetil were also important risk factors, as they alter the natural immunity to *Candida spp*. These drugs were used as a therapy for transplant recipients, neurosurgery patients and for patients with auto immune diseases like SLE and RPGN. Unconsciousness was another risk factor, as most of the patients were on prolonged indwelling catheter and prolonged

antibiotics. **Transplant was an important risk factor and it showed a statistically significant association with *C.tropicalis* Candiduria with a 'p' value of 0.002 by Chi-Square test.**

CKD was the most common disease among the diseases of urinary tract, contributing to 34% of the patient population (Table 7). It was higher than the results obtained in the study by Stephen P storfer [90], where in 19% of patients showed renal insufficiency and 18% in the study by S.Krcmery et al[5]. This increase was probably because 18 of the 34 patients were diabetic. Glycosuria and impaired functions of phagocytes in these patients are important factors favouring colonization. Uremia in CKD patients and hemodialysis in these patients, makes them more susceptible to infections. The predisposition is attributed to impairments in lymphocyte and granulocyte function, circulating inhibitors to chemotactic factors, frequent violation of skin and mucosal barriers, iron overload, underlying disorders, low albumin levels and metabolic acidosis. There was no statistically significant difference between *C.albicans* and *nonalbicans Candida spp.* in CKD patients with a 'p' value of 0.211.Other structural and anatomical abnormalities of Kidney, like Calculi, BPH, Neurogenic bladder ,RPGN ,Hypospadiasis ,Phimosis and Carcinoma prostate were found in 13% of patients , contributed to Candiduria due to the obstruction and incomplete emptying of the bladder.

Among the type of urine samples obtained, catheterized samples contributed to a total of 73% and midstream urine samples in 27% of patients.(Table 8) This was comparable to the study by Cl´audia Castelo Branco Artiaga Kobayashi et al [93] and study by Arlene O.Cantillep et al

[87]where in 84.4% and 89% of patients were catheterized. Catheterization was described by many authors as the most important risk factor.

The present study showed predominance of *non albicans Candida spp.* contributing to 86.7% of isolates and *C.albicans* contributing only 13.3% of the isolates(Table 9). This was comparable to the results obtained by Manisha jain et al[94], who showed *non albicans Candida spp*. as predominant isolate in 71.4% from urine isolates.

The species distribution was as follows, *C.tropicalis* in 63.8%, *C.albicans* in 13.3%, *C.guillermondii* and *C.krusei* in 7.8% *C.parapsilosis* in 5.7% and *C.kefyr* in 1.9% (Table 10). This was also comparable to the results by Manisha Jain et al [94] from north India. Her study showed 52.9% of isolates as *C.tropicalis* and 29.8% as *C.albicans*. In contrast, the studies by Elza Helena Da Silva et al[95] and N.Febre et al[96] from Brazil showed *C.albicans* as predominant species contributing to about 56% and 46.15% respectively. An increasing predominance of *non-albicans Candida spp.* was also noted in these studies.

Mixtures were isolated from 5 urine samples and the rest of 95 were single isolates. This was comparable to the results obtained by S.Agarwal et al [97]. *C.tropicalis* was the most common single isolate obtained, but the mixture isolates showed a predominance of *C.krusei* found in all the mixtures, followed by *C.tropicalis* (Table 11).This is a very interesting finding as *C.krusei*, more resistant species was probably replacing the original species.

Non albicans Candida spp were common in both catheterized and mid stream urine samples (Table 12). **The asscociation between *non albicans Candida spp* and catheterized patients was also highly significant with a 'p' value of 0.005 by Fischer's Exact test.** This is an important observation as 26.5% of catheter associated infections are due to fungi [98].

Biofilm formation of the *C. tropicalis* strain on the catheter surface may contribute to the colonization in patients with urinary catheter. Biofilms of *C.tropicalis*, with an extensive, hexosamine-rich matrix, were poorly penetrated by antifungal agents, where as biofilms of *C.albicans*, with a less-extensive glucose rich matrix, were more readily penetrated by drugs[99]. The exact reason for the increase in *non-albicans Candida spp.* is incompletely understood. *C.albicans* was found in 31.3% of the midstream samples but it contributed to only in 6.6% in catheterized patients. ***C.albicans* was more common in midstream urine samples than catheterized patients and this association was highly significant with a 'p' value of 0.03 by Chi-Square test.**

Assimilation was the best method of *Candida* speciation as it identified all the *Candida spp*.(100%) and was taken as gold standard for species identification [91], followed by fermentation. The ability of Hi-Chrom agar to identify the species varied greatly from 86.5% to 100% in different species(Table 13).Although there was no statistically significant difference between Hi-Chrom agar and assimilation with a 'p' value of 0.421, sensitivity of Hi-Chrom agar, the ability to identify true positive isolates varied between species. It was 100% sensitive for *C.parapsilosis*, *C.krusei*, 92.8% sensitive for

C.albicans, less sensitive for *C.tropicalis* (86.5%) and *C.guillermondii* (87.5%) (Table 14). Over all sensitivity of Chrom agar was 87.6%. This was comparable to the results of Uma Chaudary et al[91], who showed Hi-Chrom agar sensitivity of 88% -100% in different species of *Candida*. In contrast, V.P.Baradhkar et al[49], showed the sensitivity of Hi-Chrom agar for *C.tropicalis* was 100% compared to 86.5% in our study, probably because, large number of *C.tropicalis* isolates were screened in our study.

Specificity, the ability of colours to identify true negative by Hi-Chrom agar was 100% for *C.parapsilosis*, C.krusei, *C.guillermondii* and *C.kefyr* ,92.8% for *C.albicans* and 97.4 % for *C.tropicalis*. These results were comparable to the study by V.P.Baradkar et al [49], who showed a specificity of 96.4% for *C.albicans*, 100% for *C.tropicalis*, 80% for *C.parapsilosis.* The specificity for *C.parapsilosis* was higher in our study and studies on large number of isolates of *C.parapsilosis* will probably solve the reasons for such discrepancies.

Light green colonies was only 65% predictive for *C.albicans* ,as it gave a lot of false positives. 98.3% of steel blue was predictive of *C.tropicalis*. Cream colour, dry pink colonies and pale pink to purple colour was 100% predictive for *C.parapsilosis*, *C.krusei* and *C.guillermondii* .(Table 15)

In the present study, Fluconazole resistance was observed in 22.4% of *C.tropicalis* and 14.3% of *C.albicans* isolates by MBD and DD(Table 16).This was comparable to the results obtained in the study by Ariane Bruder-Nascimento et al[100], who showed resistance in 18.4% of isolates of *C.tropicalis* and Rizvi MW et al[101] who showed 20% of resistant strains of

C.albicans. But *C.albicans* was highly sensitive in study by Ariane Bruder-Nascimento et al[100], contrasting to the present study. Minor error, due to discrepancies between DD and MBD ,the SDD isolates becoming sensitive or vice-versa were observed in 3.8%, which is allowable error(Table 18). A. L. Barry et al [102] also showed minor error of 5.7% in his study.

Itraconazole sensitivity was observed in 79.1% of isolates MBD, while only 72.4% of isolates were susceptible by DD (Table 17). *C.krusei* and *C.tropicalis* were more resistant to Itraconazole than other species. This was comparable to the results by Ariane Bruder-Nascimento[100] et al, who showed 72.2% isolates were sensitive, with *C.tropicalis* being the most resistant. Minor error of 11.4% and major error of 1.9% had occurred, due to discrepancies between MBD and DD (Table 19). This shows that MBD was more reliable for Itraconazole.

All the isolates were susceptible by MBD to Amphotericin B, but (table 18) DD showed that only 87.5% of isolates were susceptible and it was due to major error of 13.3%. As it is not allowable, MBD should be relied for Amphotericin B susceptibility. Similar results were obtained in a study by Ariane Bruder-Nascimento et al. [100]

SUMMARY

- A total of 100 patients were included in the study, of which 27% of patients were in the > 60 yrs group, followed by 19% of patients in 40-49 and 50-59 yrs age group.
- Males constituted 56% and females 44% of the total population.
- The types of presentation varied as follows, asymptomatic in 42%, symptomatic in 27% and unconsciousness (patients unable to voice their symptoms) in 31% of patients.
- Fever (16%) and dysuria (11%), were the common symptoms in patients with Candiduria.
- Prolonged antibiotics (82%), catheterization (73%), Diabetes mellitus (43%), CKD(34%) were the common risk factors associated. Catheterization is a significant predisposing factor for *nonalbicans Candida spp.* Candiduria, **'p' =0.03 by Fisher's Exact test.**
- Transplant was a significant risk factor for *C.tropicalis* Candiduria, **'p'=0.002 by Chi-Square test.**
- Catheterized samples constituted 73% and mid-stream urine constituted 27% of total urine samples.
- *C.albicans* constituted 13.3% and *nonalbicans Candida spp.* constituted 86.7% of the total isolates obtained.
- *C.tropicalis* was isolated in 63.8%,*C.guillermondii & C.krusei* in 7.6%,*C.parapsilosis* in 5.7%,*C.kefyr* in 1.9% of the 105 isolates.

- 95% of samples yielded single isolates and 5% of samples yielded mixture isolates. *C.krusei* was the predominant isolate found in combination with other *Candida spp* in all the mixture.
- Sugar assimilation, though laborious was the best method of speciation followed by sugar fermentation which takes longer time to interpret. Hi-Chrom agar was a comfortable rapid method, though the sensitivity was 87.6%. It had a high positive predictive value for steel blue color indicative of *C.tropicalis*, which is the most common species isolated.
- Resistance to Fluconazole was seen in 24.7% of isolates, with *C.tropicalis* being the most resistant (68.7%).
- 79.1% isolates were sensitive to Itraconazole.
- 100% of the isolates were sensitive to Amphotericin B.
- DD can be used for Fluconazole, as minor error of only 3.8% occurred due to discrepancies between DD and MBD.
- MBD is the best method for finding out Antifungal Susceptibility for Itraconazole & Amphotericin B, as major errors occurred due to discrepancies between the methods.

CONCLUSION

100 hospitalized patients in RAJIV GANDHI GOVERNMENT GENRAL HOSPITAL, both males and females with Candiduria colony count of 10^4 were included in the study and 105 *Candida Spp* were isolated. It showed a predominance of *non albicans Candida spp* of about 86.7%. *C.tropicalis* (63.8%) was the most common isolate obtained followed by *C.albicans* (13.3%), *C.krusei* (7.6%), *C.guillermondii* (7.6%), *C.parapsilosis* (5.7%) and *C.kefyr* (1.9%). *C.krusei*, intrinsically resistant to Fluconazole was commonly isolated from mixtures. Indwelling urinary catheter was an important associated risk factor for *nonalbicans* Candiduria. Multiple risk factors like antibiotic therapy, prolonged catheterization, Diabetes, CKD and hemodialysis were present in many patients.

Laborious procedure of sugar assimilation precludes its use. Hi-Chrom agar takes only 48 hrs for species identification, and is a comfortable alternative to conventional methods, that take 96-120 hrs. Hi-chrom agar is superior to other conventional methods available for detection of mixtures. It is good especially for urine samples, as steel blue color of *C.tropicalis*, the predominant isolate, has a positive predictive value of 98.3%. It was also 100% sensitive for *C.krusei*, the most resistant *Candida* isolate. 24.7% isolates were resistant to Fluconazole and 12.4% to Itraconazole. But the positive predictive

value of light green color is only 65%, which can be overcome by combination with sugar assimilation for improving the efficiency of species identification.

Constant surveillance of Candiduria is important as *C.tropicalis* is more invasive, can lead to fatal Candidemia. Identification of *Candida* to species level is essential, as it can give an idea to the Clinicians about empirical therapy in emergency situations. Hi-Chrom agar helps in reducing the time for diagnosis, thereby reducing the duration of hospital stay and the cost of treatment. The rising antifungal resistance also demands Anti- Fungal Susceptibility testing a routine in all Microbiology Laboratories due to alarming increase of resistant fungal infection.

PROFORMA

- Name : IP no:
- Age: Ward:
- Sex:
- Occupation:
- Address
- Presenting complaints:
- Risk factors of the patients:

Underlying illness

Diabetes mellitus

Hypertension

Uremia

Any structural abnormality in urinary tract

Neoplasm

Coma

Pronged stay in ICU

Broad spectrum antibiotic, antifungal & its duration

Immunosuppressive therapy/Chemotherapy

Other candidiasis (oral/oesophageal etc)

Sepsis

Procedures

Duration & reason of catherization

Any invasive procedure in urinary tract

Transplant

Prior surgery

- ¤ Physical examination:
- ¤ laboratory evaluation:

Microbiological investigation:

Specimen:URINE

- Direct mount:
- Gram's stain:
- Culture on SDA:
- Quantitative culture:
- Germ tube:
- Culture on Chrom Agar:
- Culture on Cornmeal Agar:
- Biochemical reactions:
- Sugar fermentation: Dextrose ,Lactose, Sucrose, Maltose
- Sugar assimilation: Dextrose ,Lactose, Sucrose, Trehalose, Raffinose
- Antifungal susceptibility pattern:

  -Disk diffusion: Amphotericin B, Itraconazole & Fluconazole

  -Microbroth dilution technique : Amphotericin B, Itraconazole & Fluconazole

ABBREVIATIONS

AIDS	-	Acquired Immunodeficiency Syndrome
ATCC	-	American Type Culture Collections
BPH	-	Benign Prostatic Hypertrophy
Ca	-	Carcinoma
CLSI	-	Clinical Laboratory Standards Institute
CIE	-	Counter immune Electrophoresis
CLED agar	-	Cystine Lactose Electrolyte Deficient agar
DD	-	Disk Diffusion Method
DMSO	-	Dimethyl Sulfoxide
DNA	-	Deoxy Ribonucleic Acid
ELISA	-	Enzyme Linked Immune Sorbent Assay
EUCAST	-	European Committee for Antimicrobial Susceptibility Testing
HIV	-	Human Immunodeficiency Virus
ICU	-	Intensive Care Unit
ID	-	Immuno-diffusion

LA	-	Latex Agglutination
LPCB	-	Lacto Phenol Cotton Blue
MBD	-	Microbroth Dilution Technique
MHA	-	Mueller Hinton Agar
MIC	-	Minimum Inhibitory Concentration
MOPS	-	3N-Morphilino Propane Sulphonic Acid
PCR	-	Polymerase Chain Reaction
QC	-	Quality Control
RNA	-	Ribo Nucleic Acid
RPGN	-	Rapid Proliferative Glomerulonephritis
RPMI	-	Rosewell Park Memorial Institute
SDA	-	Sabouraud's Dextrose Agar
SDD	-	Susceptible Dose Dependent
UTI	-	Urinary Tract Infection
YNB	-	Yeast Nitrogen Base

APPENDIX

GRAM'S STAINING:

Methyl violet(2%)-10 g, Methyl violet in 100 ml absolute alcohol in 1litre of distilled water (primary stain)

Gram's iodine- 10g iodine in 20g KI (fixative)

Acetone- Decolorizing agent

Dilute Carbol Fuschin (1%)-(Secondary stain)

BLOOD AGAR:

Peptone-10gm

Nacl-5gm

Distilled water-1litre

Agar-10gm

Dissolve in distilled water by boiling. Cool to at 55 ^{o}C and add 5% sterile sheep blood . Adjust pH to 7.4.

CLED AGAR (Cystine Lactose Electrolyte Deficient Agar**)**

Peptone -4gm/l

Tryptone -4gm/l

Meat extract powder-3g/l

Lactose-10g/l

L-cystine-0.128g/l

Bromothymol blue-0.02g/l

Agar-15g/l

Water-1litre

Suspend the ingredients in water, bring to boil and dissolve. Sterilize at 121^{o}C for 15 min and mix well before pouring.

SABOURAD'S DEXTROSE AGAR WITH ANTIBIOTICS:

Peptone-10gm

Dextrose-40gm

Agar-20gm

Distilled water-1000ml

Chloramphenicol-50 mg

Final pH adjusted to 5.6

The above ingredients were reconstituted in one litre of distilled water.Dissolve the powder in distilled water by boiling.Dissolve chloramphenicol in 10 ml of 95% alcohol and added to boiling medium.The medium was then removed from heating, mixed well and then dispersed in tubes and autoclaved at 121^{o}C for 15 minutes. The final pH was adjusted to 5.6.The tubes were cooled in slanted position.

CHROM AGAR/ Hi-Chrom Candida Differential Agar:

Hi-veg special peptone-15 gm/l

Yeast extract-4gm/l

Dipotassium hydrogen phosphate-1gm/l

Chromogenic mixture-7.22gm/l

Chloramphenicol-0.50gm/l

Agar -15gm/l

pH-6.3+ 0.2

Suspend 42.72grams in 1litre of distilled water.Heat to boiling to dissolve the medium completely. Cool the medium to 50 ^{o}C and pour into sterile petri dishes.

CORNMEAL TWEEN 80 AGAR:

Cornmeal-50gm/l

Agar-15gm/l

Distilled water-1litre

Tween 80(1%) -3ml/l

Suspend the ingredients in 1 litre of distilled water and boiled to dissolve completely. Tween 80 should be added to the above medium. It is sterilized by autoclaving at 121 ^{o}C for 15 minutes.

YEAST NITROGEN BASE AGAR MEDIUM:

Ingredients	Grams/l	Ingredients	Grams/l
Ammonium Sulphate	5.00	Thiamine hydrochloride	0.004
L-Histidine	0.01	Boric acid	0.0005
Hydrochloride	0.02	Copper sulphate	0.00004
DL-Methionine	0.02	Potassium iodide	0.0001
DL-Tryptophan	0.000002	Ferric chloride	0.0002
Biotin	0.00004	Manganese chloride	0.0004
Calcium Pantothene	0.000002	Sodium molybdate	0.0002
Folic acid	0.02	Zinc sulphate	0.0004
Inositol	0.0004	Monopotassium	1.00
Niacin	0.0002	phosphate	0.50
Para amino benzoic acid	0.0004	Magnesium sulphate	0.10
Pyridoxine	0.0002	Sodium chloride	0.10
hydrochloride		Calcium chloride	
Riboflavin			

Dissolve 6.7 gms of the media in 100ml of distilled water.Sterilize by filtration and store at 4^{o}C.

CARBOHYDRATE FERMENTATION MEDIA:

Peptone- 15gm/l

Sugar to be tested-20gm/l

Nacl-5gm/l

Distilled water-1litre

Dissolve the peptone, Bromothymol blue indicator and sodium chloride in 1000ml of distilled water and 20 gms of the sugar to be tested. Distribute approx 5 ml in sterile test tubes containing an inverted Durham's (gas production). Sterilize by autoclaving at 10 pounds pressure.

MUELLER HINTON AGAR

Beef infusion-300ml

Casein hydrolysate-17.5gm

Starch-1.5gm

Agar-10gm

Distilled water-1000ml

pH-7.4

Sterilize by autoclaving 121^{o}C for 20 minutes.

RPMI 1640(ROSEWELL PARK MEMORIAL INSTITUTE) MEDIA:

RPMI MEDIUM-10.4gm

MOPS buffer-34.43gm

Dissolve powdered medium in 900ml distilled water. Add MOPS to a final concentration of 0.165 mol/L and stir until dissolved. While stirring, adjust the pH to 7.0 at 25^{o}C. Add additional water to bring medium to a final volume of 1000ml. Filter sterilize and store at 4 ^{o}C.

X Mc farland's 0.5 turbidity standard

- Prepare this turbidity standard by adding 0.5ml of 1.175% Bacl2 to 99.5ml of H2SO4 with constant stirring to maintain a suspension.
- Verify the correct density of the turbidity standard by using a spectrophotometer. The absorbance at 625nm should be 0.08 to 0.10 for the 0.5Mc Farland standard.
- Distribute 4 to 6 ml into screw capped tubes and tightly seal the tubes and store them in the dark at room temperature.
- Vigorously agitate this turbidity standard on a mechanical vortex just before use.

SUGAR ASSIMILATION PATTERN:

SPECIES	**D**	**L**	**S**	**C**	**R**
Candida tropicalis	+	-	+	+	-
Candida albicans	+	-	+	-	-
Candida parapsilosis	+	-	+	-	-
Candida krusei	+	-	-	-	-
Candida guillermondii	+	-	+	+	+
Candida kefyr	+	+	+	+(v)	+
Candida glabrata	-	-	-	-	-

(Fisher and Cook-Fundamentals of Diagnostic Mycology)

D-Dextrose, L-Lactose, S-Sucrose ,C-Cellobiose, R-Raffinose

SUGAR FERMENTATION PATTERN:

SPECIES	**G**	**L**	**M**	**S**
Candida tropicalis	+	-	+	+
Candida albicans	+	-	+	-
Candida parapsilosis	+	-	-	-
Candida krusei	+	-	-	-
Candida guillermondii	+	-	-	+
Candida kefyr	+	-	+	+
Candida glabrata	+	-	-	-

(Jagdish Chander-Textbook of Medical Mycology)

G-Glucose, L-Lactose, M-Maltose, S-Sucrose

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AGE	SEX	WARD	SPP	STATUS	catheterized/NOT	diabetic/NOT	antibiotic/NOT	immunosupp/NOT	CKD/ARF/NO CKD	RISK FACTOR	CHROM AGAR	DD-FLU	MBD-FLU	DD-ITRA	MBD-ITRA	DD-AMP	MBD-AMPHO
27	f	ortho	mix(kru+trop)	symptomatic	catheterized	not diabetic	antibiotic	no immunosupp	arf		CORRECT	R+S	R+S	S+S	S+S	R+S	S+S
24	f	nephro	guillermondii	symptomatic	catheterized	not diabetic	antibiotic	no immunosupp	arf		CORRECT	S	S	S	S	S	S
48	f	med	parapsilosis	unconscious	catheterized	diabetic	antibiotic	no immunosupp	arf	death	CORRECT	S	S	S	S	S	S
34	m	nephro	tropicalis	asymptomatic	catheterized	not diabetic	no antibiotic	no immunosupp	arf		CORRECT	S	S	S	S	S	S
23	f	icu	tropicalis	unconscious	catheterized	not diabetic	antibiotic	immunosupp	CKD		CORRECT	SDD	SDD	SDD	S	S	S
68	m	nephro	tropicalis	unconscious	catheterized	diabetic	antibiotic	no immunosupp	CKD		CORRECT	R	R	R	R	R	S
37	f	nephro	parapsilosis	unconscious	catheterized	not diabetic	antibiotic	immunosupp	CKD		CORRECT	S	S	S	S	S	S
65	f	icu	tropicalis	unconscious	catheterized	diabetic	antibiotic	no immunosupp	CKD	pyelonephritis	CORRECT	S	S	S	S	S	S
43	m	med	tropicalis	unconscious	catheterized	diabetic	no antibiotic	immunosupp	CKD		WRONG	S	S	S	S	S	S
70	m	med	tropicalis	asymptomatic	catheterized	diabetic	no antibiotic	immunosupp	CKD		CORRECT	SDD	SDD	S	S	S	S
52	f	med	tropicalis	asymptomatic	midstream urine	diabetic	antibiotic	no immunosupp	CKD	nephropathy	CORRECT	R	R	R	R	R	S
75	m	med	tropicalis	asymptomatic	midstream urine	diabetic	antibiotic	no immunosupp	CKD	diabetic nephropathy	CORRECT	R	R	SDD	S	S	S
67	m	surgery	tropicalis	symptomatic	catheterized	diabetic	antibiotic	no immunosupp	CKD		CORRECT	S	S	S	S	S	S
36	m	urology	guillermondii	symptomatic	catheterized	not diabetic	antibiotic	immunosupp	CKD	transplant	CORRECT	S	S	S	S	R	S
58	m	med	krusei	unconscious	catheterized	diabetic	antibiotic	no immunosupp	CKD	hep b +	CORRECT	R	R	S	S	S	S
54	m	nephro	mix(kru+kefyr)	asymptomatic	catheterized	not diabetic	antibiotic	immunosupp	CKD	transplant	COR+WRO	R+S	R+S	R+S	R+S	S+S	S+S
48	f	med	tropicalis	asymptomatic	midstream urine	diabetic	no antibiotic	no immunosupp	CKD		CORRECT	S	S	S	S	S	S
69	m	urology	tropicalis	symptomatic	catheterized	diabetic	antibiotic	no immunosupp	CKD	calculi,bph	CORRECT	S	S	S	S	S	S
19	m	nephro	albicans	symptomatic	midstream urine	not diabetic	antibiotic	immunosupp	CKD	transplant	CORRECT	R	R	S	S	S	S
17	f	urology	tropicalis	asymptomatic	catheterized	not diabetic	antibiotic	no immunosupp	CKD	renal TB	CORRECT	S	S	S	S	R	S
36	f	nephro	guillermondii	asymptomatic	catheterized	not diabetic	antibiotic	immunosupp	CKD	transplant	CORRECT	S	S	S	S	S	S
59	m	med	tropicalis	asymptomatic	catheterized	not diabetic	antibiotic	no immunosupp	CKD	fournier's gangrene	CORRECT	SDD	S	S	S	S	S
69	f	nephro	tropicalis	asymptomatic	midstream urine	not diabetic	no antibiotic	no immunosupp	CKD		WRONG	S	S	S	S	S	S
51	m	nephro	tropicalis	asymptomatic	midstream urine	diabetic	antibiotic	no immunosupp	CKD	hep b +,af	CORRECT	R	R	SDD	S	S	S
43	m	nephro	guillermondii	asymptomatic	midstream urine	not diabetic	no antibiotic	no immunosupp	CKD		CORRECT	S	S	R	R	S	S
72	m	surgery	tropicalis	symptomatic	midstream urine	diabetic	antibiotic	no immunosupp	CKD	pyelonephritis	WRONG	S	S	S	S	S	S
65	f	med	kefyr	unconscious	catheterized	diabetic	antibiotic	no immunosupp	CKD		WRONG	S	S	S	S	S	S
32	m	urology	krusei	asymptomatic	midstream urine	not diabetic	no antibiotic	no immunosupp	CKD	transplant	CORRECT	R	R	R+S	SDD+S	S	S
49	f	med	tropicalis	asymptomatic	midstream urine	not diabetic	no antibiotic	no immunosupp	CKD		CORRECT	S	S	S	S	S	S
69	m	nephro	tropicalis	symptomatic	midstream urine	not diabetic	antibiotic	immunosupp	CKD	transplant	CORRECT	R	R	SDD	S	R	S
37	f	nephro	albicans	asymptomatic	midstream urine	not diabetic	antibiotic	no immunosupp	CKD		CORRECT	S	S	R	R	S	S
60	m	nephro	parapsilosis	symptomatic	midstream urine	diabetic	antibiotic	no immunosupp	CKD	uti	CORRECT	S	S	S	S	S	S
28	m	nephro	tropicalis	symptomatic	midstream urine	diabetic	no antibiotic	no immunosupp	CKD		CORRECT	S	S	S	S	S	S
58	f	urology	krusei	asymptomatic	catheterized	not diabetic	antibiotic	immunosupp	CKD	transplant	CORRECT	R	R	S	S	S	S
65	f	med	tropicalis	unconscious	catheterized	diabetic	antibiotic	no immunosupp	CKD		CORRECT	SDD	SDD	S	S	R	S
46	f	surgery	mix(kru+alb)	asymptomatic	catheterized	diabetic	no antibiotic	no immunosupp	CKD	DKA	CORRECT	R+S	R+S	S+S	S+S	R+R	S+S
18	m	neurosur	tropicalis	unconscious	catheterized	not diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
72	m	med	albicans	symptomatic	midstream urine	diabetic	no antibiotic	no immunosupp	no CKD		CORRECT	S	S	R	SDD	S	S
23	m	neurosur	tropicalis	unconscious	catheterized	not diabetic	antibiotic	immunosupp	no CKD		WRONG	R	R	R	R	S	S
29	m	med	tropicalis	asymptomatic	catheterized	not diabetic	antibiotic	no immunosupp	no CKD		CORRECT	R	R	R	SDD	R	S
72	m	urology	tropicalis	symptomatic	catheterized	not diabetic	antibiotic	no immunosupp	no CKD	BPH	WRONG	S	S	S	S	S	S
38	f	nephro	albicans	symptomatic	midstream urine	not diabetic	antibiotic	immunosupp	no CKD	RPGN	CORRECT	S	S	S	S	S	S
41	m	med	tropicalis	unconscious	catheterized	not diabetic	antibiotic	immunosupp	no CKD	SLE	CORRECT	SDD	SDD	SDD	S	S	S
62	m	med	tropicalis	asymptomatic	catheterized	diabetic	antibiotic	immunosupp	no CKD	HT	CORRECT	S	S	S	S	S	S
27	f	neurosur	tropicalis	unconscious	catheterized	not diabetic	antibiotic	immunosupp	no CKD	ICH	CORRECT	R	R	R	R	S	S
39	m	neurosur	tropicalis	unconscious	catheterized	not diabetic	antibiotic	immunosupp	no CKD		CORRECT	S	S	S	S	S	S
25	f	icu	tropicalis	symptomatic	catheterized	not diabetic	antibiotic	no immunosupp	no CKD	pyelonephritis	CORRECT	S	S	S	S	S	S
27	f	med	tropicalis	asymptomatic	midstream urine	not diabetic	no antibiotic	no immunosupp	no CKD	b/l angiomyolipoma	CORRECT	S	S	S	S	S	S
26	m	neurosur	tropicalis	symptomatic	catheterized	not diabetic	antibiotic	immunosupp	no CKD		CORRECT	R	R	S	S	S	S
19	m	icu	parapsilosis	unconscious	catheterized	not diabetic	antibiotic	immunosupp	no CKD	bladder	CORRECT	SDD	SDD	S	S	R	S
28	m	icu	parapsilosis	unconscious	catheterized	not diabetic	antibiotic	immunosupp	no CKD		CORRECT	S	S	S	S	S	S
39	f	urology	parapsilosis	asymptomatic	catheterized	not diabetic	antibiotic	no immunosupp	no CKD	donor nephrectomy	CORRECT	S	S	S	S	S	S
75	f	med	tropicalis	symptomatic	midstream urine	diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
55	m	surgery	albicans	asymptomatic	midstream urine	not diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
69	m	nephro	albicans	symptomatic	midstream urine	diabetic	antibiotic	no immunosupp	no CKD	calculi	WRONG	S	S	SDD	S	S	S

50	f	med	guillermondii	symptomatic	midstream urine	not diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
42	m	surgery	tropicalis	asymptomatic	catheterized	not diabetic	antibiotic	no immunosupp	no CKD		WRONG	S	S	S	S	S	S
7	m	ortho	tropicalis	asymptomatic	catheterized	not diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	R	R	S	S
34	f	med	tropicalis	asymptomatic	midstream urine	not diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
26	f	icu	albicans	unconscious	catheterized	not diabetic	antibiotic	no immunosupp	no CKD	surgery	CORRECT	S	S	S	S	S	S
35	f	neurosur	tropicalis	unconscious	catheterized	not diabetic	antibiotic	immunosupp	no CKD		CORRECT	S	S	S	S	S	S
55	m	surgery	mix(kru+trop)	asymptomatic	catheterized	not diabetic	antibiotic	no immunosupp	no CKD		CORRECT	R+S	R+S	S+S	S+S	S+S	S+S
47	m	surgery	tropicalis	symptomatic	catheterized	diabetic	antibiotic	no immunosupp	no CKD	hypospadiasis	CORRECT	R	R	R	SDD	S	S
27	f	icu	tropicalis	unconscious	catheterized	not diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
29	f	icu	albicans	unconscious	catheterized	not diabetic	antibiotic	immunosupp	no CKD	death,antenatal	CORRECT	SDD	S	S	S	S	S
67	m	med	tropicalis	unconscious	catheterized	diabetic	antibiotic	no immunosupp	no CKD	death,bph	CORRECT	S	S	R	SDD	S	S
58	m	surgery	tropicalis	asymptomatic	catheterized	diabetic	antibiotic	no immunosupp	no CKD		CORRECT	SDD	S	S	S	S	S
22	f	icu	tropicalis	unconscious	catheterized	not diabetic	antibiotic	immunosupp	no CKD	antenatal	CORRECT	S	S	S	S	S	S
39	m	surgery	albicans	asymptomatic	catheterized	not diabetic	antibiotic	no immunosupp	no CKD	surgery	CORRECT	S	S	SDD	S	R	S
25	f	surgery	tropicalis	unconscious	catheterized	not diabetic	antibiotic	immunosupp	no CKD	HIV	CORRECT	S	S	S	S	S	S
28	f	icu	tropicalis	asymptomatic	catheterized	diabetic	antibiotic	no immunosupp	no CKD	DKA	CORRECT	R	R	R	R	S	S
70	f	med	tropicalis	asymptomatic	catheterized	diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
68	f	med	tropicalis	unconscious	catheterized	diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
43	f	surgery	tropicalis	asymptomatic	catheterized	not diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
42	f	med	albicans	symptomatic	midstream urine	diabetic	antibiotic	no immunosupp	no CKD	calculi,cystitis	CORRECT	SDD	S	S	S	S	S
58	m	urology	guillermondii	asymptomatic	catheterized	not diabetic	antibiotic	immunosupp	no CKD	transplant,af	CORRECT	R	R	S	S	S	S
49	f	surgery	tropicalis	unconscious	catheterized	not diabetic	antibiotic	no immunosupp	no CKD		WRONG	S	S	S	S	S	S
67	m	med	guillermondii	symptomatic	catheterized	diabetic	antibiotic	no immunosupp	no CKD	uti	WRONG	S	S	S	S	R	S
58	m	urology	tropicalis	symptomatic	catheterized	not diabetic	no antibiotic	no immunosupp	no CKD	bph	CORRECT	S	S	S	S	S	S
51	m	med	mix(kru+trop)	asymptomatic	catheterized	diabetic	no antibiotic	no immunosupp	no CKD		CORRECT	R+S	R+S	R+S	R+S	R+S	S+S
50	m	med	albicans	symptomatic	midstream urine	diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	R	SDD	S	S
25	m	med	tropicalis	asymptomatic	catheterized	diabetic	no antibiotic	no immunosupp	no CKD	DKA	WRONG	S	S	R	SDD	S	S
41	m	med	tropicalis	unconscious	catheterized	diabetic	antibiotic	no immunosupp	no CKD		CORRECT	R	R	R	SDD	S	S
31	m	med	tropicalis	asymptomatic	catheterized	not diabetic	antibiotic	no immunosupp	no CKD	neurogenic bladder	WRONG	S	S	S	S	S	S
69	m	med	albicans	asymptomatic	catheterized	diabetic	antibiotic	no immunosupp	no CKD		CORRECT	R	R	R	R	S	S
44	f	nephro	tropicalis	symptomatic	midstream urine	diabetic	antibiotic	no immunosupp	no CKD	pyelonephritis	CORRECT	S	S	S	S	S	S
32	m	icu	tropicalis	unconscious	catheterized	not diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
60	f	med	tropicalis	asymptomatic	catheterized	diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
73	f	geriatrics	tropicalis	asymptomatic	catheterized	not diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	R	R	S	S
43	m	icu	tropicalis	unconscious	catheterized	diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
23	f	urology	tropicalis	symptomatic	catheterized	not diabetic	antibiotic	immunosupp	no CKD	rpgn	CORRECT	R	R	R	R	S	S
49	m	ortho	tropicalis	asymptomatic	catheterized	not diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
45	m	med	tropicalis	asymptomatic	midstream urine	not diabetic	antibiotic	immunosupp	no CKD		CORRECT	S	S	S	S	S	S
44	f	med	tropicalis	asymptomatic	midstream urine	diabetic	no antibiotic	no immunosupp	no CKD		CORRECT	R	R	S	S	S	S
65	m	surgery	tropicalis	symptomatic	catheterized	diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
54	f	med	tropicalis	unconscious	catheterized	diabetic	antibiotic	no immunosupp	no CKD		CORRECT	S	S	S	S	S	S
65	m	geriatrics	albicans	symptomatic	catheterized	not diabetic	no antibiotic	no immunosupp	no CKD	ca prostate	CORRECT	S	S	S	S	S	S
45	m	surgery	tropicalis	asymptomatic	catheterized	diabetic	antibiotic	no immunosupp	no CKD	bph	CORRECT	R	R	R	SDD	S	S
76	m	med	guillermondii	unconscious	catheterized	not diabetic	no antibiotic	no immunosupp	no CKD	dcld	CORRECT	S	S	S	S	S	S
37	f	rheum	tropicalis	unconscious	catheterized	diabetic	antibiotic	immunosupp	CKD	SLE	CORRECT	S	S	S	S	S	S

"A STUDY ON URINARY ISOLATES OF *CANDIDA SPECIES* ISOLATED FROM HOSPITALIZED PATIENTS WITH SPECIAL REFERENCE TO SPECIATION, ANTIFUNGAL SUSCEPTIBILITY AND COMPARISON OF RAPID AND CONVENTIONAL METHODS OF SPECIATION"

Abstract

INTRODUCTION:

Candida are a part of human microbial flora .Although ubiquitous in nature, they can also cause various infections primarily in hospitalized patients. They are the most prevalent opportunistic fungal pathogens involving urinary tract, presenting mostly as nosocomial infection rather than community acquired infection.10-15% of nosocomial urinary tract infections are due to *Candida* and it also correlates with invasive disease significantly. As there is a sudden increase of *non-albicans Candida spp*, the present study was conducted to determine the species of *Candida* isolated from the urine of hospitalized patients, to correlate risk factors associated and to find out the antifungal susceptibility of the *Candida species* isolated.

AIMS AND OBJECTIVES:

- To speciate *Candida* isolates from urine of hospitalized patients , correlate the risk factors associated and to find Antifungal Susceptibility pattern.
- To compare the rapid method like Chrom agar for speciation with conventional methods of speciation.

MATERIALS AND METHODS:

The study was done on Hospitalized Patients both males and females, older than 12 yrs of age with urine colony count of any *Candida species* more than or equal to 10^4/ml. After, primary isolation of *Candida spp*. on Sabouraud's Dextrose agar, they were speciated using chrom agar, cornmeal agar, sugar fermentation and sugar assimilation tests. Antifungal susceptibility was done by Kirby-Bauer's disk diffusion method and microbroth dilution technique and interpreted according to CLSI guidelines.

RESULTS:

Majority of patients belonged to above 60yrs group (27%) , as they had more risk factors than others.73% of Candiduria patients were catheterized, and catheterization promoted the colonization of *non-albicans Candida species* significantly. Prolonged antibiotics and catheterization were the most common risk factors, Diabetes was the most common disease as glycosuria contributed to their colonization.

C.tropicalis contributed to 63.8%, followed by *C.albicans* (13.3%) but mixtures showed a predominance of *C.krusei,* a highly resistant species.Hi- Chrom agar was able to identify 87.5% of the isolates. It has huge advantage of early speciation and simple technique, hence can be done for routine speciation combined with conventional technique whenever discrepancies arise.24.8% of isolates were resistant to Fluconazole and 12.4% to Itraconazole. All the isolates were susceptible to Amphotericin B.

CONCLUSION:

Constant surveillance of Candiduria and eradication is important as it is more invasive, leading to fatal Candidemia. Identification of *Candida* to species level can give information to Clinicians about empirical therapy. Hi-Chrom agar , helps in reducing time for diagnosis, thereby reducing duration of hospital stay and cost of treatment. The rising antifungal resistance also demands Anti- Fungal Susceptibility testing a routine in Microbiology Laboratories.

KEY WORDS: Candiduria, *non-albicans Candida species,* Chrom agar ,Anti- Fungal Susceptibility.